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VOL. 26 MAY, 1955 No. 3

Original Articles

METICORTEN AND 9 ALPHA FLUOROHYDROCORTISONE IN THE TREATMENT OF ALLERGIC DISORDERS

CARL E. ARBESMAN, M.D., AND ROBERT J. EHRENREICH, M.D., BUFFALO, N. Y.

THE symptomatic relief of many allergic disorders, including severe bronchial asthma, with the various steroids, cortisone, hydrocortisone, and the corticotrophins, already has been well established. This very definite advance has certain limitations and drawbacks. The side effects, or rather the hyperphysiologic effects, of these substances often necessitate either the complete withdrawal of the compound or a reduction in the dosage. This reduction in dosage usually reduces the ill effects, but also decreases its beneficial action. Hence, any steroid which would relieve allergic symptoms without producing side effects would be a decided advance in therapy for the allergic patient.

Many investigators have worked on this problem and several synthetically prepared and related steroids have been studied. Two of these steroids have been made available for clinical trial. It is the purpose of this article to report the findings on these two products, namely, 9 alpha fluorohydrocortisone and Meticorten (metacortandracin).

9 ALPHA FLUOROHYDROCORTISONE

Fried and Sabo[1] found that the 9-alpha-halogen derivatives of hydrocortisone were very active in the rat liver assay for eleven oxygenated corticosteroids. Goldfien and his associates[2] studied these derivatives in man and concluded that 9 alpha fluorohydrocortisone (the insertion of a fluorine atom at the 9 position of hydrocortisone) had much greater metabolic activity and duration of action than hydrocortisone. They also reported that sodium retention and

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Meticorten was furnished through the courtesy of the Schering Corporation, Bloomfield, New Jersey.

9 alpha fluorohydrocortisone was furnished through the courtesy of E. R. Squibb & Sons, New York City.

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potassium diuresis occurred with as little as 0.25 mg. of a single dose of this product, and that an Addisonian patient had been maintained on the same dosage (0.25 mg.) daily.

Method.—In order to evaluate properly this steroid and compare its clinical effectiveness with cortisone or hydrocortisone, seven patients with severe asthma from private practice, who had previously responded well to the other steroids and had been on standardized maintenance dosages with these steroids for long periods of time, were switched to 9 alpha fluorohydrocortisone. One other patient, without previous steroid therapy, also was treated with this compound and included in the results.

The patients were advised to continue on a sodium-poor diet and had supplemental potassium, in the form of either potassium iodide or chloride prescribed. They were observed at least once weekly. The dosage schedule was usually 1 mg. of 9 alpha fluorohydrocortisone for each 25 mg. tablet of cortisone or 20 mg. tablet of hydrocortisone.

Results.—The abbreviated case histories of these patients are listed below and a summary is shown in Table I.

TABLE I. PATIENTS TREATED WITH 9 ALPHA FLUOROHYDROCORTISONE
SUMMARY OF RESULTS

NAME	AGE	DOSAGE MG. PER DAY	NUMBER OF DAYS USED	RESULTS	SIDE EFFECTS	BLOOD CHEMISTRY		
						SODIUM	POTASSIUM	CALCIUM
M. V.	35	8	4	Aggravated	Edema, infection			
M. S.	71	4	6	Aggravated	Edema, twitching, salt taste, restlessness	147	3.3	
						143	3.3	
						144	4.6	4.3
						144	4.1	4.3
						137	3.9	4.2
G. L.	57	4	7	None	None			
		12	6					
L. C.	52	8	4	None	Edema, bloating, moon face	pre 140	5.1	
						post 150	4.4	
E. S.	57	3	6	None	Edema, bloating, moon face	151	4.6	
E. S.	66	2	14	None	Edema	150	3.6	
M. F.	63	3	7	Aggravated	Salt taste, drowsiness, irritability, edema	138	5.1	
H. R.	56	8	1	None	None			
		4	3					

CASE 1.—M. V., a 35-year-old woman, has had severe bronchial asthma and allergic rhinitis since early childhood. Despite specific hyposensitization with the many extrinsic factors to which she was allergic, bronchodilators, and the other usual methods of therapy, she did not respond well. Under cortisone and hydrocortisone therapy, in a maintenance dosage of 75 or 60 mg., respectively, she improved somewhat, but not completely. While under this fair control, the other steroid was stopped and 9 alpha fluorohydrocortisone was prescribed. She received 2 mg. four times daily the first day, and then 1 mg. four times daily for the next three days. She developed pain in the chest, fever, and edema on the third day, and required hospitalization. Heroic measures, including oxygen, antibiotics, intravenous aminophylline, etc., were necessary for this patient during a stormy four-week stay in the hospital.

CASE 2.—M. S., a 71-year-old woman, had severe asthma for nine years. Injection therapy with inhalants, plus self-administered adrenalin injections, and general symptomatic measures along with hydrocortisone kept her fairly comfortable. On Sept. 11, 1954, the hydrocortisone was stopped and 1 mg. of 9 alpha fluorohydrocortisone, four times daily, was prescribed. Her asthmatic symptoms got progressively worse, she developed marked edema, muscular twitchings, weariness, and a dry, salty taste in her mouth. Her condition became critical and she was hospitalized on Sept. 16, 1954. The medication was stopped immediately; diuretics were administered, along with supplemental potassium in large doses, and water ingestion was encouraged. Symptomatic measures for relief of her asthma, such as aminophylline, epinephrine, and oxygen, were employed. After a stay of eighteen days in the hospital, the edema and other symptoms subsided. The patient was discharged on hydrocortisone improved. The blood chemical studies done during her hospital stay are shown in milliequivalents:

Date	*Sodium*	*Potassium*	*Calcium*	*Bicarbonate*
9/17/54	147	3.3		
9/18/54	143	3.3		
9/23/54	144	4.6	4.3	
9/25/54	144	4.1	4.3	26.2
9/29/54	137	3.9	4.2	

Several electrocardiograms showed myocardial asthenia and dipping of the R-T segments which suggest muscle hypoxia on Sept. 23, 1954. On Sept. 29, 1954, the only finding was that of low voltage in limb leads; otherwise the tracing was normal.

CASE 3.—G. L., a 57-year-old man, has had multiple recurrent nasal and sinus polyps, severe allergic rhinitis, and bronchial asthma for many years. Under treatment with specific hyposensitization and maintenance therapy of ACTH, cortisone, and hydrocortisone for the past three and one-half years, he has progressed very well. He was able to return to work and get along comfortably as long as he took the steroid therapy. He was switched to 4 mg. of 9 alpha fluorohydrocortisone daily for one week. His nasal and bronchial symptoms increased. The dosage of this steroid was increased to 12 mg. daily for six more days, but his symptoms continued to get worse, although he did not develop any edema. Cortisone, 25 mg. three times daily, was reinstituted and within forty-eight hours his symptoms were well controlled.

CASE 4.—L. C., a 52-year-old man with severe intrinsic bronchial asthma, is aspirin-sensitive; his condition has been fairly well controlled on 60 to 70 mg. of hydrocortisone daily for over two years. His steroid was changed to 8 mg. of 9 alpha fluorohydrocortisone for four days. His asthma increased; he developed a marked moon face, had bloating, gained 4 pounds in weight, and was very uncomfortable. His blood chemistry prior to the administration of this new compound was serum sodium 140 milliequivalents and serum potassium 5.1. However, after three days and a total of only 24 mg. of 9 alpha fluorohydrocortisone, the serum sodium was 150 and the potassium was 4.4. Diuretics and the readministration of 60 to 70 mg. of hydrocortisone daily soon had this man back to his "normal."

CASE 5.—E. S., a woman aged 57 years, has had severe chronic intrinsic asthma for many years and is aspirin-sensitive. On 40 to 60 mg. of hydrocortisone, her symptoms were fairly well controlled for two to three years. She was given 1 mg. of 9 alpha fluorohydrocortisone three times daily for six days. Her asthma got much worse; she developed marked ankle edema and bloating, and gained 7 pounds in weight. Her serum sodium was 151 milliequivalents and potassium was 4.6. On mercurial diuretics and hydrocortisone, her edema and symptoms subsided.

CASE 6.—E. S., a 66-year-old man, has had severe intrinsic chronic bronchial asthma for ten years. He had been on maintenance dosages of either 25 to 50 mg. cortisone or 30 mg. of hydrocortisone for three and one-half years, with fairly good results. On Sept. 6, 1954, he was transferred to 2 mg. of this new steroid daily. He felt bloated, gained 3 pounds in one

week, and his asthmatic symptoms became much worse, although he claimed that he felt stronger. His serum sodium and potassium were 150 and 3.6, respectively, on Sept. 25, 1954. Like the others, with the administration of mercurials and a return to hydrocortisone, he returned to his previous status.

CASE 7.—M. F., a 63-year-old woman, has had severe extrinsic asthma for twenty years. Symptoms were fairly well controlled with specific hyposensitization and either cortisone or hydrocortisone for three years. The maintenance dosage was 25 to 37½ mg. of cortisone daily. She was transferred to 1 mg. of 9 alpha fluorohydrocortisone three times daily for one week. Her asthma became very severe; she had a marked salty taste in her mouth, marked drowsiness irritability, and nervousness. She gained 3 pounds in weight. Unfortunately, her blood chemistry was not studied until eight days after stopping this steroid. The results at that time were: serum sodium, 138 milliequivalents, and potassium, 5.1. Upon reapplication of the hydrocortisone therapy, she soon returned to her former condition.

One other patient was tried on this substance.

CASE 8.—H. R., a man aged 56 years, had seasonal ragweed hayfever and asthma and, despite adequate specific hyposensitization, antihistaminics, and other symptomatic measures, his symptoms continued. He was given 9 alpha fluorohydrocortisone, 8 mg. the first day and 4 mg. daily for two more days, without any appreciable benefit or ill effects.

As can be seen from the table and the case reports, this new steroid is of no value in the relief of symptoms of bronchial asthma, but it does have powerful sodium-retaining properties. This characteristic action may be the cause of the alarming aggravation of asthmatic symptoms in some of the patients. Although the systemic use of 9 alpha fluorohydrocortisone is not beneficial in allergic disorders, it has been reported to be of value in the treatment of Addison's disease,[2] and also its topical application is of great benefit in various allergic dermatoses.[3, 7]

METICORTEN (METACORTANDRACIN)

This compound is one of a new series of synthetically prepared crystalline corticosteroids. Its chemical formula is $\triangle^{1,4}$-pregnadiene-17 α, 21-diol-3, 11, 20-trione. It was produced by dehydrogenation at positions 1 and 2 of the cortisone nucleus, resulting in the double bond between C_1 and C_2. This seemingly small alteration in structure changed its action considerably. Bunim, Pechet, and Bollet[4] reported prompt falls in the urinary 17-ketosteroids and reduction in the number of circulating eosinophils with this compound. Prolonged use of Meticorten did not cause sodium retention in the usual beneficial therapeutic dosages for the treatment of patients with arthritis.

One of the most frequent and disturbing side effects of steroid therapy in the allergic patients is that of marked fluid retention, weight gain, and sodium increase. This is particularly noticeable in patients who receive either cortisone or hydrocortisone after a course of ACTH.[5] Hence, if the results obtained in the arthritic patients could be duplicated in the allergic individual, Meticorten would indeed be a boon to the asthmatic sufferers.

Method.—Meticorten was studied in twenty patients. Seventeen of these twenty patients had been on maintenance therapy of either cortisone, hydrocortisone, or ACTH for long periods of time. Five of these seventeen patients

also had had 9 alpha fluorohydrocortisone. The dosage prescribed to these seventeen patients was, roughly, the substitution of 5 mg. of Meticorten for each 25 mg. of cortisone and 20 mg. of hydrocortisone. The drug was administered in four equally divided doses. At the beginning of this study, sodium-poor diets and supplemental potassium were recommended, but this was soon discontinued. In addition to these patients, three others received Meticorten. The initial dosage was usually 5 mg. four times daily for several days, then gradual reduction to the point of minimal dosage but adequate clinical response.

All these patients were seen at least once weekly. Examinations of the chest and heart, weight, and blood pressure were recorded, as were the patients' subjective remarks. Determinations of the serum sodium and potassium levels and absolute eosinophil counts were also obtained. Patients were given just enough medication to last them until their next visit.

Results.—

I. Fifteen of the sixteen patients, all of whom had had previous steroid therapy for their asthma, obtained at least equally beneficial results from Meticorten (Table II). One man, H. V., aged 54 years, was symptomatically controlled on 30 to 40 mg. of hydrocortisone. He was switched to 10 mg. of Meticorten and developed severe epigastric pain which required hospitalization. Complete studies, including upper gastrointestinal and gall bladder x-rays, stool examinations, blood chemistry, and blood counts, all failed to yield any positive findings. Meticorten was reinstituted and again the severe pain recurred. The patient was switched back to hydrocortisone without difficulty or ill effects, and he has remained well (eight weeks).

TABLE II. METICORTEN

DIAGNOSIS	NUMBER OF CASES	RESULTS		
		GOOD	FAIR	POOR
Bronchial asthma	5*	5		
	11	10		1
Without previous steroids (bronchial asthma)	1	1		
Dermatomyositis	1	1		
Perennial allergic rhinitis	1			1
Chronic urticaria and angioedema	1			1

*Previously treated with 9 alpha fluorohydrocortisone.

An additional patient, Mrs. M. C., aged 38 years, a physician's wife, has had a positive muscle biopsy and diagnosis of dermatomyositis. She was reasonably well controlled on 40 mg. of hydrocortisone. She was transferred to 15 mg. of Meticorten daily for the past seven to eight weeks. Her symptoms are much better controlled; she has no edema, which was very prevalent on hydrocortisone, less pain, and a general feeling of well-being.

II. Of the three patients who had not had previous steroid therapy, one, J. R., a 20-year-old patient with severe asthma, obtained excellent relief with

TABLE III

NAME	AGE	SEX	DURATION OF PREVIOUS STEROID THERAPY	PREVIOUS STEROID USED	AVERAGE MAINTENANCE DOSAGE PER MG.	METICORTEN		
						DURATION† OF TREATMENT IN WEEKS	AVERAGE MAINTENANCE DOSAGE PER MG.	RELATIVE EFFECTIVENESS
1. H. V.	54	M	3 months	Hydrocortisone	30	6/7	10	Worse epigastric pain
2. H. J.	73	F	6 weeks	Cortisone	25	9	5	Same
3. M. B.	63	F	8 months	Cortisone	25 to 50	7	5 to 7.5	Better
4. T. K.	63	M	3 years, 8 months	ACTH gel	5 U. every other day	6	7.5 to 10	Better
				Hydrocortisone	20 to 40			
				Cortisone	25 to 50			
5. B. F.	48	F	4 weeks	Cortisone	25 to 50	2	10	Better
6. J. P.	34	F	21 weeks	Cortisone	50	3	15	Better
7. G. C.	55	M	2 weeks	Hydrocortisone	60	8	10 to 15	Better
8. A. D.	60	F	3 months	Cortisone	50 to 75	10	7.5 to 10	Better
9. E. Z.	47	F	3 months	Hydrocortisone	30	9	5	Same
				Cortisone	75			
10. A. Z.	42	M	2 months	Cortisone	50 to 100	5	10 to 15	Better
11. H. E.	59	M	1½ years	Cortisone	50	9	7.5	Better
12. G. L.*	56	M	3 years, 2 months	Cortisone	50 to 75	8	15	Better
				Hydrocortisone	40 to 60			
13. M. F.*	63	F	3 years, 6 months	Cortisone	50	5	7.5	Same
				Hydrocortisone	20 to 40			
14. E. S.*	61	F	3 years	Hydrocortisone	40 to 60	8	15	Better
15. E. S.*	67	M	3½ years	Cortisone	25 to 50	8	7.5	Same
				Hydrocortisone	20 to 40			
16. L. C.*	53	M	4 years plus	Cortisone	75 to 100	9	15 to 20	Better
				Hydrocortisone	60 to 70			
17. M. C.	38	F	3 years	Hydrocortisone	40	6	15	Better

*Previously treated with 9 alpha fluorohydrocortisone.

†Since submitting these data for publication, all but one of the patients have continued to use Meticorten for an additional three weeks.

an initial daily dosage of 20 mg. for one week; after adjusting his dosage each week, it was found that 10 to 15 mg. daily was his maintenance level. He has been on Meticorten for ten weeks thus far.

Another patient, a man aged 40 years, with severe perennial allergic rhinitis, despite 20 mg. Meticorten daily for two weeks, failed to respond. The third patient was a 12½-year-old girl suffering from generalized giant urticaria and angioneurotic edema who, likewise, did not benefit from 20 mg. Meticorten daily. These results are summarized in Table II.

III. *Duration of treatment, comparative dosage, and effectiveness:* Although the length of time which these patients have received Meticorten is relatively short as compared with prolonged therapy with the other steroids, certain preliminary comparisons can be pointed out. The details of the duration of treatment of the various steroids in each patient, as well as the average maintenance dosage, are given in Table III. This new steroid appears to be at least

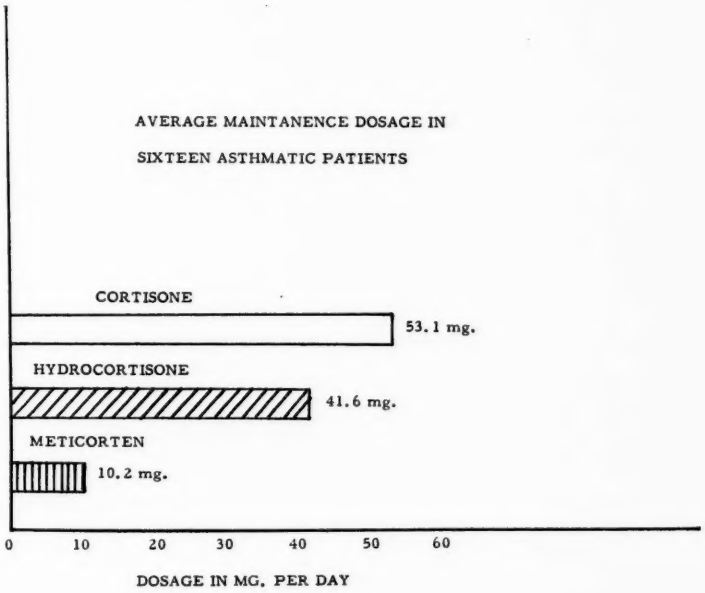

Fig. 1.

four to five times more potent than cortisone and three to four times as effective as hydrocortisone, compared milligram for milligram. It has been shown previously[6] that the dosage of hydrocortisone is approximately two-thirds that of cortisone. The actual average daily maintenance dosage of these sixteen asthmatic patients treated with Meticorten was 10 to 15 mg. (10.2 mg.); for cortisone, 50 to 75 mg. (53.1 mg.); and for hydrocortisone, 40 to 60 mg. (41.6 mg.) (Fig. 1). This dosage varied not only from patient to patient, but even in the same patient under different circumstances.

TABLE IV. COMPARISON OF STEROIDS FOR WATER RETENTION

	EDEMA	
NUMBER OF PATIENTS	CORTISONE OR HYDROCORTISONE	METICORTEN
17	6	1

Six of the seventeen patients, when previously treated with either cortisone or hydrocortisone, developed marked water retention and weight gain. All but one of these six patients lost their fluid retention when treated with Meticorten (Table IV). Four of the seventeen patients claimed that Meticorten and the other steroids were equally beneficial, whereas twelve patients felt that Meticorten was more effective (Table V).

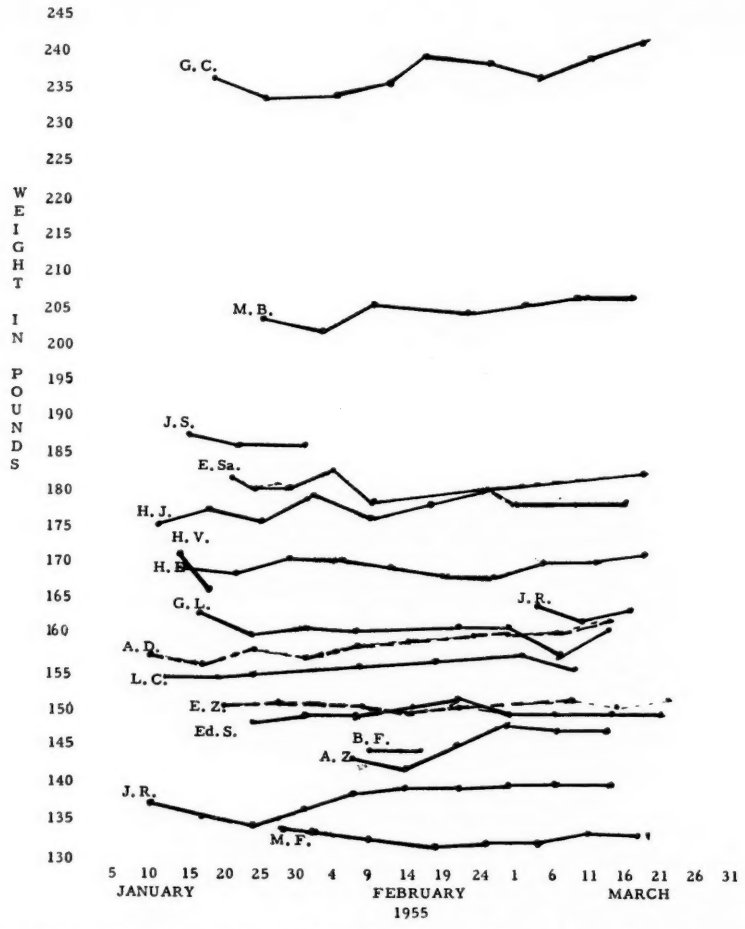

Fig. 2.—Graph showing the actual weights of the patients at each office visit while under Meticorten therapy. No significant weight gain was observed.

IV. *Effect of Meticorten on weight, electrolytes, eosinophils, and blood pressure:* The simplest method of determining water and sodium retention is

TABLE V. COMPARATIVE EFFECTIVENESS

NUMBER OF PATIENTS	CORTISONE OR HYDROCORTISONE BETTER	EQUAL	METICORTEN BETTER
17	1	4	12

TABLE VI

NAME	LENGTH OF TREATMENT IN WEEKS	AVERAGE MAINTENANCE DOSAGE PER MG.	BLOOD PRESSURE		CHANGE IN WEIGHT (POUNDS)	SODIUM (MEQ.)	POTASSIUM (MEQ.)	ABSOLUTE EOSINOPHIL COUNT (C.MM.)
			BEFORE TREATMENT	AFTER TREATMENT				
1. J. R.	11	10 to 15	105/70	110/76	+2	137		90
2. L. C.*	10	15 to 20			=	138	4.1	
						137		
3. E. S.*	9	7.5	160/90	160/80	+2	138	4.6	75
4. E. S.*	10	15			+4	145		240
						141	4.6	22
5. M. F.*	7	5	140/70	138/70	-1½	142	5.6	
						134	4.9	
6. G. L.*	10	10 to 15	115/90	110/70	-5	128	5.9	
7. H. E.	9	7.5	134/84	130/80	-1	144		220
							3.9	20
8. A. Z.	8	10 to 15	110/70	100/60	+5	139	4.9	15
								60
9. E. Z.	9	5	140/90	154/94	+1½	142	4.9	
						140	4.4	
10. A. D.	11	7.5	155/80	145/86	+3½	147		1100
						143	5.4	
11. G. C.	10	15	120/84	110/70	+3	142		
						138		
12. T. K.	7	7.5			+4½	148	5.6	130
						142	4.4	
13. M. B.	7	5	190/130	160/110	+3	140	4.1	24
14. H. J.	9	2.5 to 7.5		200/120	+3	145	5.1	
15. H. V.	6/7	10			=	144	3.9	638
								308
16. J. P.	4/7	10			-2			
17. B. F.	3	10	120/90	120/80	=			
18. G. S.	2	15 to 20			-1			
19. J. S.	1	10 to 20	100/70	100/70	-¾			
20. M. C.	6	15			-3			

*Previously treated with 9 alpha fluorohydrocortisone.

by a careful observation of the body weight. These patients were weighed at least once weekly. A chart of each patient's weights is illustrated in Fig. 2. As can be noted, there was very little increase in weight in the length of time the patients had been observed. Six patients gained 3 to 5 pounds without any evidence of edema; three patients' weight remained the same, five lost weight; and the remaining five showed a minor weight increase of 2 pounds or less (Table VI).

Serum sodium and potassium levels, as well as absolute eosinophil counts, were determined on all patients who had been on Meticorten for six or more weeks. In no instance was there an abnormally increased amount of serum sodium or decrease in potassium values (Table VI), this despite the fact that patients were not on a low-sodium diet or given supplemental potassium. In several instances, the serum potassium returned to a higher level than when patients were on the other steroids.

Several patients maintained high absolute eosinophil counts, but still had a good clinical response to Meticorten (Table VI). That there is not necessarily a correlation between eosinophil and clinical response has already been reported.[5]

There was no demonstrable effect on the blood pressures of the patients treated with Meticorten. Two patients with severe hypertension and bronchial asthma had a slight reduction in their blood pressure levels after being adequately treated with Meticorten.

DISCUSSION

The results described would indicate that Meticorten is superior to both cortisone and hydrocortisone in the treatment of bronchial asthma. It is not the lower dosage which is significant, but rather the lack of sodium retention or potassium loss. Whether these properties would be manifest if large doses of Meticorten were employed over a more prolonged period of time has yet to be determined.

Meticorten is not innocuous. It is still very potent, and one should closely adhere to all the usual precautions and limitations of the other steroids. The side effects observed in our series of twenty patients consisted of severe epigastric pain in one patient (as described) and transient discomfort under the left breast in two female patients. One other patient, H. E., a 59-year-old man, had been on cortisone, 25 to 50 mg. daily for eighteen months. He was switched over to 15 mg. Meticorten on Jan. 15, 1955, and was maintained on 7.5 mg. On Feb. 26, 1955, this patient complained of pain in both posterior pelvic areas and some low back pain. This continued, and an x-ray picture on March 19, 1955, revealed a *localized* decalcification of the third lumbar vertebra. All other vertebrae and bony structures show no evidence of osteoporosis. Further studies will be made to determine whether this is the result of the prolonged cortisone therapy, the Meticorten, or some other etiological factor. (The usual findings of osteoporosis in prolonged steroid therapy is much more general-

ized.) A chart of the various studies on this patient is illustrated in Fig. 3. This is typical of the data obtained in other patients studied; that is, little or no effect on the body weight, blood pressure, and blood chemistry.

The more beneficial results with Meticorten over the other steroids in the majority of these patients may well be a question of dosage, rather than efficacy. In all probability, this and other modifications of the corticosteroids eventually may lead to a substance which will have all the desirable effects and

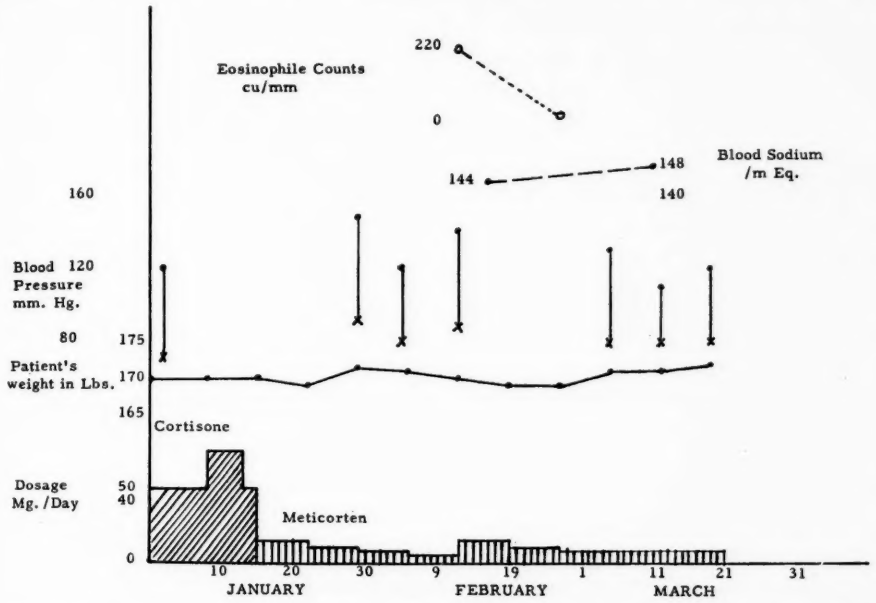

Fig. 3.—Data gathered on patient H. E. This is one typical example of the results obtained in all the other patients.

none of the ill effects of the present steroids. It is entirely conceivable that organic chemists will be so successful in this search that cortisone and hydrocortisone will no longer be used.

SUMMARY AND CONCLUSIONS

1. Two new steroids were studied for clinical effectiveness in allergic disorders.

a. 9 alpha fluorohydrocortisone, although very effective for sodium retention and eosinophil reduction response, was of no value in the relief of asthmatic symptoms.

b. Meticorten gave beneficial results in the fifteen asthmatic patients who previously had obtained relief with other steroids. An additional asthmatic patient without previous steroid therapy also felt much improved on this new compound. Dermatomyositis was more satisfactorily controlled by Meticorten than by hydrocortisone in one patient. One patient with severe perennial allergic rhinitis and another with giant urticaria and angioedema failed to respond to the usual dosages prescribed as previously explained.

2. Meticorten, despite no supplemental potassium or sodium restriction, did not affect these electrolytes in patients with the dosages used in this study.

3. Meticorten did not affect the blood pressure or body weight.

4. Meticorten is clinically four to five times as effective as cortisone and three to four times as effective as hydrocortisone, milligram for milligram.

5. These are preliminary reports, as the long-term effect of this new steroid must yet be determined. The usual precautions and contraindications of the other steroids should be observed.

6. Meticorten, like the other steroids, is not a substitute for specific management of the allergic or asthmatic patient.

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METACORTANDRACIN* IN CHRONIC ASTHMA

A Preliminary Report

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METACORTANDRACIN, a new synthetic corticosteroid for oral therapy, has recently been made available for general use. Structurally, it is similar to cortisone and hydrocortisone, as illustrated in Fig. 1. In a preliminary report by Bunim, Petchet, and Bollet[1] it was felt that metacortandracin possessed antirheumatic and anti-inflammatory effects similar to metacortandralone which, in turn, seems more effective than hydrocortisone, the latter itself being about 1.5 times more effective, milligram for milligram, than the original cortisone. The side effects in their patients, all of whom had rheumatoid arthritis, were minor and often disappeared as the dose of the drug was decreased.

The purpose of this report is to present our observations on a very small group of patients with severe chronic asthma who have recently responded poorly to corticotropin, cortisone, and hydrocortisone, and have now been on metacortandracin for a short period of time. We feel that this report is justified, as this drug is now available commercially and may be useful in controlling those asthmatic patients who respond poorly to other corticosteroids or cannot tolerate the preparations because of side effects. Careful balance studies have not yet been done.

Response to therapy of a patient with chronic athma is difficult to evaluate in accurately measurable terms. In evaluating our patients, we relied on changes in symptomatology, physical findings, and vital capacities. Five patients were treated on an outpatient basis and three were hospitalized. Antibiotics and supportive therapy were also used as indicated.

The initial dose of metacortandracin was 15 to 50 mg. daily, divided into four equal parts, and modified thereafter according to the patient's response. That all patients responded under therapy was apparent, although the response in some consisted of stabilizing a rapidly deteriorating asthmatic state. Because of the many factors that enter into the response of any asthmatic patient to specific medication, it is possible that this dosage may be inadequate in some instances or too large in others. The periodicity of dosage and total daily requirements are still under study.

From The Institute of Allergy, The Roosevelt Hospital, New York City.
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*This drug, under the name Meticorten (now known as Prednisone), was supplied through the courtesy of the Schering Corporation, Bloomfield, New Jersey.

CASE REPORTS

CASE 1.—S. N., a 39-year-old boat captain, has had chronic infective asthma for about twenty years. Complete allergic investigation revealed no significant inhalant or food factor. During the past two years he has been hospitalized five times for acute asthmatic episodes, usually following upper respiratory infections. Each admission showed a slower and less effective response to corticotropin and antibiotics.

On the last two admissions, this patient required from 25 units of ACTH daily, intravenously, to 80 units of ACTH gel daily, which resulted in moderate improvement but also in a ten-pound weight gain. Two months after the last hospitalization, he developed moderately severe asthma and was placed on corticotropin gel, 40 units daily, and tetracycline on an outpatient basis for one week. He continued a limited amount of work. There was slight improvement but, because of a six-pound gain in weight, the corticotropin was gradually reduced and discontinued. The blood pressure remained about 155/85. There were moderate rhonchi throughout both lung fields. The weight was 162½ pounds.

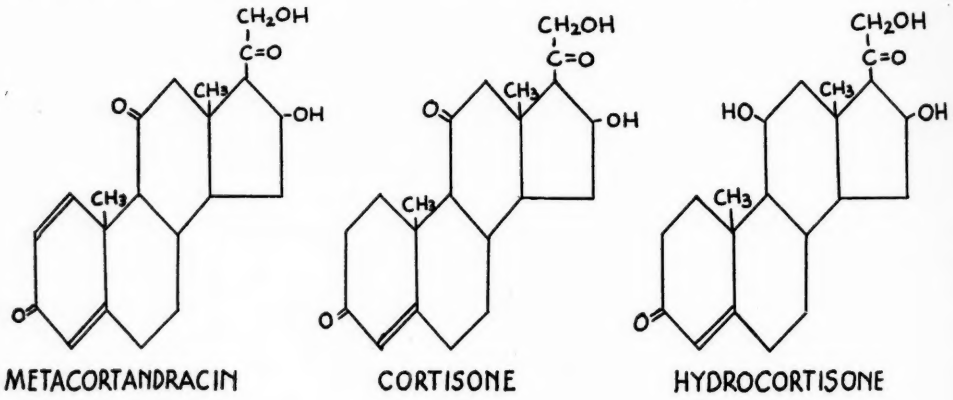

Fig. 1.

He was then placed on metacortandracin, 40 mg. daily, in divided doses. Within three days there was improvement and the dosage was reduced to 30 mg. daily. He then increased his work load, which caused a worsening of his asthma and reduced his vital capacity from an earlier 65 per cent to 48 per cent of normal.

Metacortandracin was increased to 40 mg. daily and tetracycline was added. There was considerable improvement and he continued to work while the metacortandracin was gradually reduced over an eight-day period. Four days later, however, it was necessary to increase the dosage again to 40 mg. daily. The blood pressure remained about 135/85 and the weight unchanged at 162 pounds.

CASE 2.—F. L. is a 60-year-old unemployed Negro man who has had chronic infective asthma for three years. The most recent of his ten hospital admissions have required high dosage of corticotropin and antibiotics, and the clinical response has become progressively slower.

On the tenth hospitalization he was given high dosage of corticotropin, both intravenously, 25 to 60 units daily, and in the gel form, 40 units daily. This was followed by oral hydrocortisone up to 400 mg. daily. There was considerable subjective improvement, but he continued to have moderate rhonchi throughout both lung fields. There was a ten-pound weight gain and the blood pressure increased from 145/85 to 160/100 while he was on corticotropin.

On the twenty-seventh hospital day he was placed on metacortandracin, 40 mg. daily in divided doses. Within four days there was improvement and he was discharged on 20 mg. daily. Over a nine-day period at home on this dosage, his vital capacity improved from 63 per cent to

76 per cent, but moderate rhonchi persisted. The dosage was reduced to 15 mg. daily but, because of the development of an upper respiratory infection, the asthma worsened and the vital capacity was reduced to 28 per cent of normal.

The metacortandracin was increased to 30 mg. daily and tetracycline was added. In three days the lung findings were markedly improved and the vital capacity increased to 83 per cent. After decreasing the dosage to 20 mg. daily, the vital capacity was 74 per cent, and there was a slight increase in the pulmonary signs. During the entire time on metacortandracin his blood pressure remained about 155/85 to 90 and the weight was unchanged at 192 pounds.

CASE 3.—C. L. is a 67-year-old white housewife who has had infective asthma for twelve years. She was admitted recently to The Roosevelt Hospital for the eleventh time because of moderately severe asthma, which did not respond to a normally therapeutic dose of corticotropin gel, 40 units daily, on an outpatient basis.

In the hospital she responded very slowly to intravenous corticotropin, corticotropin gel, antibiotics, and supportive therapy. She was placed on hydrocortisone, 100 mg. daily, for four days and she continued to improve, but slowly. Her blood pressure remained 150/85 and her weight 164½ pounds. On the seventeenth hospital day she was placed on metacortandracin, 40 mg. daily, while there were still sibilant sounds in the right lung. In two days both lungs were clear and the patient was discharged on 20 mg. of metacortandracin daily.

The metacortandracin was gradually reduced over a fourteen-day period to 5 mg. daily, during which time she was free of asthma. The blood pressure and weight remained unchanged.

CASE 4.—A. C., a 40-year-old white housewife who has had infective asthma for eleven years, was discharged from The Roosevelt Hospital on Jan. 18, 1955, after being treated with antibiotics, corticotropin, and supportive therapy. On previous cortisone therapy, 50 to 75 mg. daily, she had improved, but had a moderate weight gain. She did well until the middle of February, when she again became asthmatic. She was seen in the clinic on Feb. 25, 1955, having moderately severe asthma. The vital capacity was 2,600 c.c. (81 per cent of normal); weight, 141 pounds; and the blood pressure, 110/80. Metacortandracin, 30 mg. daily, was started on this date. On March 1, 1955, she was improved; there was no weight gain and the blood pressure remained normal. The metacortandracin was reduced to 15 mg. daily. On March 8, 1955, she was found to have minimal sibilant sounds in the chest and the sputum was purulent. Because of this, an antibiotic was added. When last seen on March 11, 1955, she had improved considerably. The blood pressure and weight had remained normal, and the vital capacity had increased to 3,200 c.c. (103 per cent of normal). She is being maintained on 15 mg. of metacortandracin daily with similar effect but no weight gain.

CASE 5.—W. C., a 61-year-old white sanitation engineer who has had asthma due to dust, feathers, and infection for twenty-six years, was admitted to The Roosevelt Hospital on Feb. 18, 1955. He had been on maintenance cortisone therapy, 12.5 mg. daily, since May, 1954. He was admitted primarily for repair of a right inguinal hernia which had recently become incarcerated. However, because of severe paroxysms of coughing and wheezing at night, it was necessary that his medical condition be improved prior to surgery. On admission he was moderately asthmatic and was given hydrocortisone, 60 mg. daily, antibiotics, and a low salt (2 Gm.) diet. The admission weight was 186 pounds and the blood pressure was normal. On the second hospital day he showed pedal edema and, in spite of a gradual decrease in the hydrocortisone dosage, the weight increased to 190¾ pounds. On Feb. 23, 1955, the hydrocortisone was discontinued and metacortandracin, 30 mg. daily, was substituted. There was no change in the diet or antibiotics. On this dosage the asthma was well controlled and the weight returned to normal. The hernia was repaired on March 1, 1955. The wound healed well and the postoperative course was uneventful. He was discharged on metacortandracin. His weight and blood pressure remained normal.

CASE 6.—A. W. is a 63-year-old machine operator who has had severe asthma due to dust, feathers, dander, and infection for nine years. Because of fibrocalcific lesions in both upper lung fields, steroid therapy had been held to a minimum in the past. He had been on cortisone, 12.5 mg. twice daily, for the preceding week. His blood pressure was 160/90;

weight 164; and vital capacity, 1,800 c.c. The cortone was discontinued March 10, 1955, and metacortandracin, 15 mg. daily, and Gantrisin, 0.5 Gm. twice daily, were prescribed. He was again seen on March 14, 1955, and was having acute asthma precipitated by rushing to the clinic. There was no essential change in condition. He was given adrenalin-in-oil (1-500), 1 ml., and the metacortandracin was increased to 20 mg. daily. His blood pressure was 160/98 and his weight remained the same. This dosage has been maintained at this level and the patient has been able to resume work, even though he continues to have early morning asthma requiring ephedrine. There has been no evidence of fluid retention or blood pressure elevation.

CASE 7.—B. G., a 60-year-old white salesman, has had asthma with respiratory infection since age 10. When he was 25 years old he developed early and late hayfever. In 1931 he underwent bilateral radical antral surgery because of polyps and infection. By 1941 he was having hayfever, pollen asthma, and asthma associated with infection. In 1942 hemoptysis appeared and the only cause that could be discovered was a small ulceration in the left main bronchus. This has persisted intermittently to date. He has been treated in the last several years with corticosteroids, symptomatic medication, antibiotics, and pollen desensitization. He was seen on March 17, 1955, having severe asthma and hemoptysis requiring ephedrine three to five times a day and adrenalin by inhalation twenty times daily. His blood pressure was 160/104; weight, 155½ pounds; and vital capacity, 29 per cent of normal. Metacortandracin, 30 mg. daily, was prescribed. After four days he no longer needed ephedrine and used his adrenalin inhalator about one-half as frequently. He has had no more hemoptysis and the blood pressure and weight remained at the original levels. There was no evidence of pedal edema. The dose was gradually decreased to 20 mg. daily in divided doses. However, he began to notice a little more asthma and he has been continued on 30 mg. daily to date.

CASE 8.—F. B. is a 38-year-old white housewife who has had chronic infectious asthma for seven years. She has been hospitalized more than twenty times during this period for asthma. She has had numerous ethmoidal polypectomies over the years and a bilateral Caldwell-Luc operation was performed in 1950.

Prior to this hospital admission, she had been taking cortone, 75 mg. daily, and required adrenalin 1-1000, 0.4 ml. two or three times a day, in addition to four or five ephedrine tablets daily. On admission her weight was 114 pounds; blood pressure, 130/80, and fasting blood sugar, 114 mg. per 100 ml. She was given metacortandracin, 50 mg. in divided doses, for the first two days and 40 mg. for the next two days. She promptly improved and her need for ephedrine drugs decreased to once daily and the adrenalin was used only once during the night for the first three days and since then she has required no symptomatic medication. She was also given antibiotics. Her weight and blood pressure have remained stable.

From these few cases, it appears that metacortandracin is useful in the treatment of asthma. On the clinical basis of symptomatic control, the required dosage of this drug is, roughly, only one-third to one-half that of hydrocortisone.

Past experience with three of these patients has shown that the dosage of corticotropin required to bring about an equivalent clinical response was accompanied by salt retention with edema. None of these patients under metacortandracin showed evidence of fluid retention, blood pressure elevation, or glycosuria. It should be emphasized, however, that these patients have not been taking metacortandracin for more than one month at the time of this report. In the patient who underwent surgery while on this medication, there was no surgical complication and wound healing was not delayed. Where antibiotics were required with corticotropin and other corticosteroids, they were also required with metacortandracin.

The symptomatic response of these asthmatic patients to metacortandracin was essentially no different from earlier responses to corticotropin and other corticosteroids. A significant feature of this drug in bringing about this response is the relatively small dosage required. Of greater importance, however, is the apparent lack of side effects, namely, edema and blood pressure elevation, when using effective therapeutic doses in patients with severe chronic asthma. There has been no evidence of epigastric discomfort, hirsutism, facial roundness, sleeplessness, or acne in any of these patients during this short observation period.

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METACORTANDRACIN (PREDNISONE) IN BRONCHIAL ASTHMA

Comparative Effects of Metacortandracin, Hydrocortisone, and Cortisone in Bronchial Asthma With Diabetes Mellitus and Bronchial Asthma With Nasal Polyps: Preliminary Report

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METACORTANDRACIN and metacortandralone, two new synthetic steroids, were subjected to clinical trial by Bunim, Pechet, and Bollet[1] in rheumatoid arthritis. Their preliminary observations indicated that metacortandracin possessed the physiologic activity of an adrenal cortical hormone and that the antirheumatic potency and hormonal effects of both were similar. They showed that these drugs were several times more potent as antirheumatic and anti-inflammatory agents than cortisone or hydrocortisone and that the undesirable side effects were minor in nature and degree.

A study was undertaken to determine whether metacortandracin will influence the symptoms of intractable bronchial asthma. Metacortandracin was administered to two patients. The first had chronic bronchial asthma and diabetes mellitus and the second had chronic bronchial asthma and large nasal polyps. Both patients had previously received cortisone and hydrocortisone, and this afforded an opportunity for comparison of clinical responses and side effects. The duration of therapy with metacortandracin in Case 1 was ninety-four days and in Case 2, ninety-six days.

CASE REPORTS

Case 1.—Y. K., a 52-year-old housewife, had diabetes mellitus for nine years and bronchial asthma for three years. Her diet was calculated to consist of carbohydrate, 190 Gm.; protein, 60 Gm.; fat, 50 Gm.; total calories, 1,450. The diet was unchanged during cortisone, hydrocortisone, and metacortandracin therapy. Before steroid therapy, insulin requirements averaged 40 units daily, with an occasional increase to 50 or 60 units daily. Her weight was maintained at approximately 150 pounds.

With the onset of asthma, ten to fifteen injections of 1 c.c. of epinephrine were administered daily, with satisfactory but temporary relief of asthmatic symptoms. As a result of the frequent injections of epinephrine, the glycosuria increased and insulin requirements were raised to 100 to 120 units daily.

Oral cortisone was given, on and off, for a period of one year. At times the asthmatic symptoms responded well to cortisone and at other times failed to respond at all, even with large doses. It also was necessary to administer five to fifteen injections of 1 c.c. of epinephrine daily for relief of the asthma during cortisone therapy. As a result, insulin requirements increased to 150 to 200 units daily to control glycosuria. Cortisone was then dis-

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continued and, without cortisone for several months, epinephrine requirements again ranged from ten to fifteen injections of 1 c.c. daily, and insulin requirements still ranged between 150 and 200 units.

Administration of hydrocortisone for a period of three months afforded considerable relief of her asthma. As a result, the epinephrine requirements dropped to two to three injections of 0.5 c.c. daily and insulin requirements dropped to between 100 and 120 units daily, with the same dietary pattern as previously.

Metacortandracin was then administered with an initial daily dose of 25 mg. in divided doses, one 5 mg. tablet after each meal and 10 mg. before bedtime. The patient noted satisfactory relief of asthmatic symptoms one hour after the second dose. The daily dose of metacortandracin was 25 mg. for the first three days. As a result of the marked symptomatic relief, no epinephrine injections were given for the first five days. Glycosuria increased and insulin requirements were raised to 150 units on the second day of metacortandracin therapy. Metacortandracin was then decreased to 15 mg. daily and on this dose the asthmatic symptoms increased in severity, requiring one injection of epinephrine daily for three days and two injections for one day. Increasing metacortandracin gradually to 25 mg. daily as a maintenance dose, the patient again showed marked symptomatic relief of the asthmatic symptoms and no epinephrine was necessary for the following eighty-six days.

With a decrease of the dose of metacortandracin to 15 mg. daily for four days the insulin requirements dropped to 70 units daily and the asthmatic symptoms became more severe. However, with a gradual increase of the daily dose to 25 mg., the insulin requirements at this time ranged between 25 and 60 units, with an average of approximately 35 units daily for eighty-three days. Relief of the asthmatic symptoms was satisfactory and no epinephrine was necessary for eighty-six days.

Except for an increase of the glycosuria with cortisone and hydrocortisone, there were no other abnormal laboratory findings and no hormonal side effects in this patient. At the end of twelve days of therapy with metacortandracin, the blood sugar was 103 mg. per cent. There was no change in serum sodium, potassium, chlorides, cholesterol, cholesterol esters, uric acid, creatinine, calcium, and phosphorus. The patient's weight was maintained at about 150 pounds and at no time was there any evidence of hypertension. No hormonal side effects, edema, or facial rounding were observed in this patient with metacortandracin.

The comparative effects of cortisone, hydrocortisone, and metacortandracin and their relationships to epinephrine and insulin requirements are presented in Table I.

TABLE I. COMPARATIVE EFFECTS OF METACORTANDRACIN, HYDROCORTISONE, AND CORTISONE IN BRONCHIAL ASTHMA WITH DIABETES MELLITUS

THERAPY		EPINEPHRINE REQUIREMENT (INJECTIONS PER DAY)	INSULIN REQUIREMENTS (UNITS PER DAY)
Before onset of asthma	none	0	40 (occasionally 50 to 60)
	Usual therapeutic measures for 1 year	10 to 15 Dose: 1 c.c. each	100 to 120
	Cortisone 75 to 100 mg. daily for one year	5 to 15 Dose: 1 c.c. each	150 to 200
	Cortisone discontinued for 6 months.	10 to 15 Dose: 1 c.c. each	150 to 200
After onset of asthma	Hydrocortisone 60 to 80 mg. daily for 3 months.	2 to 3 Dose: 0.5 c.c. each	100 to 120
	Metacortandracin 15 to 25 mg. daily for one month.	0 for 4 days 1 to 2 for 4 days 0 for 86 days	60 first day 150 second day 120 third day 70 six days (25 to 60—83 days average—35 units)

CASE 2.—H. F., a 36-year-old housewife had chronic asthma for twenty-four years. Since the winter of 1950 her asthmatic symptoms were continuous and very severe. She also had recurrent large nasal polyps on both sides, which were removed surgically on several occasions, with recurrences. The usual methods of treatment for asthma, such as intramuscular epinephrine, intravenous aminophylline, Vaponefrin, Isuprel, and Aerolin compound by inhalation, iodides, and allergic management, etc., afforded slight and temporary relief. The patient finally resorted to the sole use of Aerolin compound by inhalation. This drug seemed to give her slightly more immediate relief than the others and the method of administration was convenient. When first seen on July 8, 1951, she had been using 50 to 100 treatments, of four or five sprays each, every day for the previous six months. She also had received five short courses of small doses of cortisone during that time, with slight relief.

In July, 1951, cortisone therapy was started again with an initial dose of 100 mg. daily and a maintenance dose of 50 to 100 mg. daily. On this dosage schedule, the asthmatic symptoms were controlled and the patient was comfortable and able to attend to her household duties. At times the nasal polyp, which was present on the right side, decreased in size. However, after ten months of continuous therapy the polyp had increased considerably in size and, in addition, another large polyp was now visible on the right side and a medium sized polyp on the left. Repeated acute upper respiratory infections occurred, requiring antibiotic therapy, and on those occasions cortisone was increased to 150 to 200 mg. daily to relieve the asthmatic symptoms. On 150 to 200 mg. of cortisone, the polyps decreased considerably in size, only to increase again on smaller dosage. Hydrocortisone was given for two and one-half years, the dosage ranging between 60 and 100 mg. The patient very often switched from cortisone to hydrocortisone and then back to cortisone in the ratio of 4:3 or 5:4. It seemed at times that the nasal polyps were slightly smaller on cortisone therapy, and on other occasions they responded better to hydrocortisone when given in larger doses. On several occasions, short courses of Acthar Gel were administered with approximately the same clinical response.

Several times during the past three and one-half years, cortisone or hydrocortisone was discontinued and replaced by the usual therapeutic measures, such as epinephrine, aminophylline, iodides, and many other drugs, and at times by no treatment at all, with a recurrence of such severe asthma that the patient requested the return of cortisone or hydrocortisone. During the past three and one-half years, the patient was on a low salt diet and potassium chloride, 3 Gm. daily. She gained 30 pounds during the three and one-half years of cortisone and hydrocortisone therapy. There were no significant changes in serum sodium, potassium, calcium, phosphorus, chlorides, creatinine, uric acid, cholesterol, or sugar levels. There was no alteration in the blood pressure on cortisone or hydrocortisone therapy.

In November, 1954, the patient slipped on a waxed floor and complained of pain in the right hip region. Radiographic examination showed a moderate, generalized demineralization of the bony trabecular structure of the dorsolumbar spine, pelvis, and hip. There was evidence of a fracture of the right lower ramus of the pelvis.

The patient then received ninety-six days of metacortandracin therapy. Therapy was initiated with a daily dose of 25 mg. At first the response was slight and, on increasing gradually to a daily dose of 35 mg., the symptomatic relief was excellent, decreasing the use of the spray from fifty to one-hundred times daily to two to five times. The nasal polyps on the right side, which had produced complete nasal obstruction before metacortandracin therapy, have decreased to approximately one-sixteenth their original size and the polyp on the left side has almost completely disappeared. After eighteen days of therapy, there was evidence of increased formation of new bone over the fracture line of the right lower ramus of the pelvis and a moderate, generalized demineralization of the spine and pelvis was still present.

On metacortandracin there were no changes in laboratory findings, and no undesirable side effects were encountered.

SUMMARY AND CONCLUSIONS

This is a preliminary report on the favorable response of two patients with bronchial asthma to the administration of metacortandracin. The duration of therapy was ninety-four days in one patient and ninety-six days in the second patient. Metacortandracin was three to four times more potent as an antiallergic drug than cortisone, and two to three times more potent than hydrocortisone. In the patient with asthma and diabetes mellitus, glycosuria was less and insulin requirements were lower on metacortandracin than on cortisone or hydrocortisone. The decrease may be due to the fact that more satisfactory relief of the asthmatic symptoms was obtained with metacortandracin resulting in the elimination of epinephrine injections which previously had increased the glycosuria. Perhaps to a lesser extent the decrease in glycosuria also may be due to a lesser effect of metacortandracin on carbohydrate metabolism than cortisone or hydrocortisone. In the second patient there was considerable decrease in size of the nasal polyps and shrinkage was much more effective with metacortandracin than with cortisone or hydrocortisone in this patient. No undesirable side effects were observed with metacortandracin. At the present time one cannot predict the results and effects from prolonged administration.

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A METHOD FOR THE OBJECTIVE EVALUATION OF BRONCHODILATOR DRUGS

Effects of Dapanone, Isuprel, and Aminophylline in Patients With Bronchial Asthma

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INTRODUCTION

SEVERAL years ago we undertook the evaluation of a new bronchodilator agent, 3,4-dihydroxyisopropylamine propiophenone hydrobromide (Dapanone†) in patients with bronchial asthma. At first we attempted to perform fairly complete pulmonary function studies on the patients before and after administration of various bronchodilators. However, it soon became apparent that it was difficult, if not impossible, to evaluate and compare several agents using a large number of pulmonary function tests because (1) the tests consume so much time that the condition of the patient may change spontaneously before the tests are completed, (2) multiple tests performed in rapid succession fatigue the patient and make the data less reliable, and (3) multiple tests require expensive equipment and trained laboratory personnel.

It was decided, therefore, to repeat the studies on another series of patients, using only one test which meets several requirements:

A. The test measures objectively the degree of bronchial or bronchiolar obstruction. In patients with such obstruction, three different situations may exist: (1) some airways are obstructed completely, while others are normal; (2) all airways are obstructed to a certain extent, but none is completely closed off; and (3) some are closed off completely, while others are narrowed or partially obstructed. In the first and third situations, the vital capacity will be reduced, whereas in the second it will be normal if sufficient time is allowed to complete the test; in the second and third, maximal expiratory flow rate will be reduced, whereas in the first it will be normal. Therefore, it seemed advisable to use a test which measures both vital capacity and maximum expiratory flow rate, rather than either alone, since the pathologic lesion in any particular patient may be predominantly either 1, 2, or 3.

This requirement eliminates the vital capacity alone as the ideal test for the measurement of bronchial obstruction, as the vital capacity is decreased only if there is complete obstruction of a significant number of airways.

The maximum breathing capacity (M.B.C.) test has been used to evaluate bronchodilator drugs,[1] but it has several defects when used for this pur-

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pose: (1) the test is so fatiguing that it is unwise to use it in severely incapacitated patients; (2) a fatiguelike effect occurs and lasts at least five minutes after a single test, thus preventing its use in serial determinations over a short space of time;[2] (3) the inertia and friction of the moving parts of a Benedict Roth or Tissot spirometer can introduce a large error in the M.B.C., and optimal results are achieved only in a relatively narrow range of respiratory rates;[3] and (4) the respiratory effort involved may induce bronchial obstruction, by either direct or reflex action.[4, 5] For these reasons, the M.B.C. test is not ideal for the testing of bronchodilator drugs.

Two tests which do meet the first requirement are the timed vital capacity and the maximal expiratory flow rate.* For reasons discussed in full elsewhere,[8] we prefer to use the second of these two tests and it was used throughout this study. Spirographic tracings have been used for years by many investigators. Our modification consists largely in the use of a very rapid kymograph, which permits actual measurement of flow rates. Essentially, our test consists of asking the patient to inspire fully and then to expire as rapidly and forcefully as possible into a well-balanced spirometer writing with minimal fric-

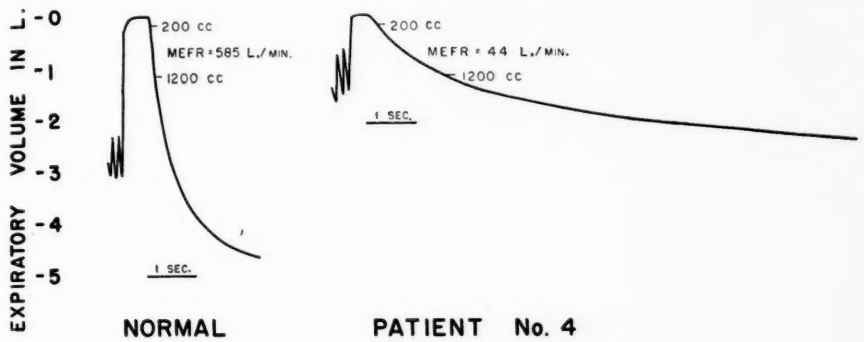

Fig. 1.—Expiratory curves of normal subject and of Patient 4.

tion on a rapidly moving kymograph. This one procedure yields values for both vital capacity and maximal expiratory flow rates (M.E.F.R.) (Fig. 1). Maximal effort is used, as it exaggerates the effect of expiratory obstruction. The maximum expiratory flow rate does not distinguish between the effects of check valves, bronchiolar constriction, mucosal congestion, mucosal edema, or secretions in the airway, but it does give a measurement of the total resistive forces during expiration.

B. The technique is rapid and simple, requiring a minimum of understanding and cooperation on the part of the patient.

C. The equipment is relatively inexpensive and does not require highly trained personnel, thus permitting the application of the technique in clinics and in the office.

*Tissue and airway resistance together has been determined by measuring air flow and alveolar pressure immediately after interruption of flow[6] and airway resistance alone has recently been measured by a body plethysmograph technique;[7] neither of these methods is considered here because each requires special equipment.

D. The test does not fatigue the patient and frequent serial measurements may be made so that the investigator can determine the onset of drug action, the time for peak action, and the duration of action. In a period of two hours it is possible to measure the effect of a drug on one patient and to determine whether one or two other drugs can produce a detectable additive effect.

PATIENTS

We studied seventeen asthmatic patients from the allergy clinic of the Hospital of the University of Pennsylvania. They ranged in age from 18 to 67 years. The duration of their disease was from one to forty-five years (Table I). On the basis of disability and of frequency and duration of attacks, all patients were judged to have severe or moderately severe asthma. All had decreased vital capacity and maximal expiratory flow rate, the latter being

TABLE I. DATA UPON PATIENTS STUDIED

	PATIENT	AGE	SEX	S. A. (M^2)	DURATION OF ASTHMA (YEARS)	FREQUENCY OF ATTACKS	SEVERITY OF DISEASE*
1	I. B.	67	M	1.84	15	Perennial—nightly	S
2	L. B.	53	M	1.63	45	Autumn and winter—one week to one month	M
3	B.Ja.**	38	M	1.76	35	Perennial—several times daily	S
4	B. Jo.***	28	M	1.86	10	Perennial—daily	S
5	C. K.	63	F	1.59	12	Perennial—daily	S
6	J. L.	65	F	1.80	31	Perennial—mild attacks daily	M
7	L. O.	48	F	1.38	14	Perennial—mild attacks daily	M
8	H. P.	44	M	1.60	16	Spring and auumn—one week to one month	M
9	J. J.***	56	M	1.59	32	Perennial—daily	S
10	J. B.	22	M	1.90	9	Daily during June and September	S
11	M. C.	24	M	1.70	19	Perennial—daily	S
12	J. H.	57	M	1.65	20	Perennial—variable	M
13	J. S. J.	18	M	1.72	15	Spring and autumn—daily	S
14	B. M.	26	M	1.84	1	Summer and autumn—daily	S
15	G. N.	65	F	1.74	6	Perennial—daily	S
16	J. T.	63	F	1.48	2	Perennial—almost daily	S
17	L. W.	48	F	2.02	22	Perennial—one month to one year	M

*M, moderately severe; S, severe.
**On maintenance digitalis for heart failure since 1951 (cor pulmonale).
***On cortisone, 25 mg. t.i.d., during all studies.

diminished to a greater extent. Two patients (Nos. 4 and 9) took cortisone daily for their asthma during the period of study. Otherwise, the patients used no bronchodilator drugs for at least twelve hours prior to a test. No patient was studied during an acute asthmatic attack. However, all patients had diffuse expiratory wheezes and prolongation of expiration, and almost all of them complained of varying degrees of exertional dyspnea.

PROCEDURE

The patient was seated. After several normal respirations, the patient's nose was occluded. He then took a maximal inspiration and expired as force-

fully, rapidly, and completely as possible through wide-bore rubber tubing (total cross-sectional area, 39.2 sq. cm.) into a carefully counterbalanced, 10 liter, Benedict Roth type spirometer, which recorded with minimal resistance on a rapid kymograph moving at 3.1 cm. per second.* The maximal expiratory flow rate was calculated from the spirogram for the liter of gas expelled between the first 200 and 1,200 c.c. of the forced expiration (see Fig. 1) and expressed in liters per minute. All volumes were corrected to B.T.P.S. (body, temperature, and pressure, saturated). The maximum of several trials was recorded. M.E.F.R. in healthy young adults is about 400 to 500 liters per minute.

After several control tests, drugs were administered. On one day, Isuprel (1:200 aerosol) was administered first, followed thirty minutes later by Dapanone (2 per cent or 5 per cent aerosol); on another day, the order was reversed. This permitted us to measure the effect of each drug alone and also to determine whether either drug produced an additional bronchodilator effect after the other presumably had achieved its maximal bronchodilator effect. In all cases, aminophylline (0.5 gram given intravenously over a ten-minute period) was administered last.

In general, the aerosols were administered over a ten-minute period, using a No. 40 DeVilbiss nebulizer; the dosage of the aerosol administered during the ten-minute period varied between 0.2 and 0.4 c.c. Vital capacity and maximal expiratory flow rate were measured at five- to ten-minute intervals during the next thirty minutes.

Two control measurements were made: (1) On one visit, measurements of vital capacity and maximal expiratory flow rate were made every five to ten minutes over a thirty-minute period after the inhalation of 0.2 to 0.4 c.c. of nebulized water, and (2) the day-by-day variations in vital capacity and maximal expiratory flow rate were measured in nine patients not given any specific bronchodilator therapy.

RESULTS AND DISCUSSION

In our studies, water administered by aerosol produced no significant changes in vital capacity or maximum expiratory flow rate over a thirty-

TABLE II. THE EFFECT OF WATER ADMINISTERED BY NEBULIZER

PATIENT	VITAL CAPACITY (CUBIC CENTIMETERS)		MAXIMAL EXPIRATORY FLOW RATES (LITERS PER MINUTE)	
	CONTROL	AFTER H_2O	CONTROL	AFTER H_2O
1	3640	4020	121	132
2	2560	2510	35	35
3	1580	1960	13	17
4	2650	2330	46	31
5	2590	2590	58	68
6	2480	2590	96	81
7	2580	2580	102	85
8	2580	2850	151	161
Mean difference	83.75		1.6	
t	0.980		0.371	
P	>.1		>.1	

*Constructed by Osborn-Blodgett Company, Havertown, Pennsylvania.

TABLE III. VARIATION FROM DAY TO DAY IN V.C. AND M.E.F.R. WITHOUT DRUG THERAPY

PATIENT NUMBER	1		2		3		4		5		6		7		8		9	
STUDY NO.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.	V.C.	M.E.F.R.
1	2610	47	2670	56	1940	20	2530	23	760	20*	2620	75	2540	120	2970	180	890	15*
2	3460	85	2560	35	1370	11	3820	79	1140	23*	2320	73	2540	119	2860	213	2580	18
		(21)		(3)		(7)		(7)		(14)		(21)		(5)		(24)		(41)
3	1780	22	2600	33	1580	13	3430	60	2230	73	2480	96	2610	111	2580	151	1340	7
		(3)		(4)		(3)		(7)		(28)		(14)		(9)		(16)		(15)
4	3640	121					3650	106	2760	103			2680	102				
		(46)						(139)		(28)				(3)				
5							2670	47	1460	10								
								(7)		(25)								
6							2650	46	2760	72								
								(18)		(41)								
7									2590	58								
										(14)								
Mean	2870	69	2610	41	1630	15	3125	60	1810	52	2470	81	2570	108	2800	181	1605	73
Maximal variation from mean in absolute figures	1090	52	60	15	310	5	695	46	1050	51	150	15	100	12	170	32	975	5
Maximal per cent variation from mean	38	75	2	37	18	33	22	78	58	98	6	19	4	11	9	18	61	38

*Measured between 200 and 700 c.c. instead of between 200 and 1200 c.c.
Figures in parentheses denote the number of days after the previous study.

minute period (Table II), but frequently the patients reported subjective relief. In addition, as observed previously,[9] there was marked day-to-day variation in the objective measurements in patients without specific therapy (Table III). In general, there was poor correlation between subjective and objective signs of improvement. These observations emphasize the problem involved in attempting to evaluate bronchodilator drugs given orally over a period of days, or of drawing conclusions from even shorter studies if objective tests are not used and each patient does not serve as his own control.

TABLE IV. EFFECT OF 2 PER CENT DAPANONE, 0.5 PER CENT ISUPREL, AND 0.5 GM. AMINOPHYLLINE, GIVEN IN THAT ORDER

	VITAL CAPACITY (CUBIC CENTIMETERS)					MAXIMAL EXPIRATORY FLOW RATE (LITERS PER MINUTE)			
PATIENT	CONTROL	AFTER 2 PER CENT DAPANONE	AFTER ISUPREL	AFTER AMINOPHYLLINE	PREDICTED V.C.*	CONTROL	AFTER 2 PER CENT DAPANONE	AFTER ISUPREL	AFTER AMINOPHYLLINE
10	1520	2050	2210	2360	4640	39	61	82	110
11	2670	3300	4140	4450	4380	50	53	85	118
12	2150	2310	2050	2360	3740	41	52	62	83
13	3980	4560	4820	4770	4190	130	111	254	365
16	1260	1570	1840	2260	2920	19	28	39	64
Mean difference		442	254	228			5.2	43.4	43.6
t		2.98	1.45	2.79			0.96	1.77	2.57
P		<0.01	>0.1	<0.05			>0.1	>0.1	<0.05

*V.C. in supine position in Tables IV to VII calculated from regression formula based on height and age.

Our data indicate that either five per cent Dapanone or 0.5 per cent Isuprel, given initially, is an effective bronchodilator drug (Tables IV, V, VI, and VII).* Each drug produces a significant increase in M.E.F.R. (though not in V.C.) if administered after time has elapsed for maximal action of the other drug, administered first. The administration of either five per cent Dapanone or 0.5 per cent Isuprel usually produced an increase in V.C. and

TABLE V. EFFECTS OF 0.5 PER CENT ISUPREL, 2 PER CENT DAPANONE, AND 0.5 GM. AMINOPHYLLINE, GIVEN IN THAT ORDER

	VITAL CAPACITY (CUBIC CENTIMETERS)					MAXIMAL EXPIRATORY FLOW RATE (LITERS PER MINUTE)			
PATIENT	CONTROL	AFTER ISUPREL	AFTER 2 PER CENT DAPANONE	AFTER AMINOPHYLLINE	PREDICTED V.C.	CONTROL	AFTER ISUPREL	AFTER 2 PER CENT DAPANONE	AFTER AMINOPHYLLINE
12	1620	2300	2460	2770	3740	29	83	96	157
14	950	1000	1110	1580	4700	92*	135*	135*	244*
15	1890	2420	2630	2780	2180	27	56	66	85
16	1670	2300	2140	2510	2110	31	57	61	83
17	1780	2040	2250	2570	2920	43	73	131	170
Mean difference		430	106	324			36.4	17	50
t		3.59	1.22	3.95			4.42	1.62	3.02
P		<0.01	>0.1	<0.01			<0.01	>0.1	<0.05

*M.E.F.R. measured from 200 to 700 c.c. instead of from 200 to 1,200 c.c.

* Five per cent Dapanone increased significantly both the vital capacity and M.E.F.R., whereas the 2 per cent solution increased significantly only the vital capacity in the patients tested.

TABLE VI. EFFECTS OF 5 PER CENT DAPANONE, 0.5 PER CENT ISUPREL, AND 0.5 GM. AMINOPHYLLINE, GIVEN IN THAT ORDER

	VITAL CAPACITY (CUBIC CENTIMETERS)					MAXIMAL EXPIRATORY FLOW RATE (LITERS PER MINUTE)			
PATIENT	CONTROL	AFTER 5 PER CENT DAPANONE	AFTER ISUPREL	AFTER AMINOPHYLLINE	PREDICTED V.C.	CONTROL	AFTER 5 PER CENT DAPANONE	AFTER ISUPREL	AFTER AMINOPHYLLINE
1	3460	3890	4320	4160	3520	85	139	147	186
2	2670	2720	2560	2720	4250	56	64	73	86
3	1940	2570	2540	2590	4250	20	34	30	33
4	3650	4470	4470	4360	2010	106	145	174	170
5	1460	1840	2000	2110	4020	10	21	22	41
6	2620	2620	2680	2780	2010	75	96	127	145
7	2540	2650	2820	2700	2830	119	133	206	214
8	2970	3190	3330	3400	3310	180	245	292	356
Mean difference	330	96.8	18.5			28.5	53	20	
t	3.36	0.71	1.79			3.94	3.80	2.61	
P	<0.01	>0.1	>0.1			<0.01	<0.01	<0.05	

M.E.F.R. immediately following the ten-minute period of aerosol administration. The peak effect occurred in almost every instance within thirty minutes following the administration of these drugs. Fig. 2 shows a response obtained in one of the asthmatic patients studied.

We conclude that the two drugs have approximately the same potency, administered as aerosols in these concentrations. Neither drug produces maximal bronchodilatation, since 0.5 gram of aminophylline, given intravenously, produced a further increase in either vital capacity or maximal expiratory flow rate, or both, in practically every patient. This suggests that the most effective way to produce bronchodilatation is to administer drugs both locally (by aerosol) and systemically. There are several reasons for this: (1) Aerosolized bronchodilators probably are unable to reach completely occluded areas of the lung, and exert their effect only on partially obstructed or patent airways; drugs administered systemically reach all bronchioles, whether completely obstructed or not, by way of the bronchial

TABLE VII. EFFECTS OF 0.5 PER CENT ISUPREL, 5 PER CENT DAPANONE, AND 0.5 GM. AMINOPHYLLINE, GIVEN IN THAT ORDER

	VITAL CAPACITY (CUBIC CENTIMETERS)					MAXIMAL EXPIRATORY FLOW RATE (LITERS PER MINUTE)			
PATIENT	CONTROL	AFTER ISUPREL	AFTER 5 PER CENT DAPANONE	AFTER AMINOPHYLLINE	PREDICTED V.C.	CONTROL	AFTER ISUPREL	AFTER 5 PER CENT DAPANONE	AFTER AMINOPHYLLINE
1	1780	3130	3190	3510	3520	22	79	87	95
2	2600	2760	2760	2760	4020	33	69	77	57
3	1370	2130	2130	2020	4250	11	27	24	20
4	2670	3710	3920	3980	4250	47	88	98	100
5	2760	3080	3190	3080	2010	72	203	243	209
6	2320	2590	2540	2640	2010	73	108	109	129
7	2610	2770	2650	2820	2830	111	154	157	217
8	2860	3080	3300	3300	3310	213	251	270	308
Mean difference	535	55	41.5			49.6	11.1	8.75	
t	3.53	1.29	0.81			4.01	2.35	0.814	
P	<0.01	>0.1	>0.1			<0.01	<0.05	>0.1	

arterial circulation. This may explain the observations that aerosols are ineffective in many patients with severe asthma and that their effectiveness increases once the airways are opened partially by adrenalin or aminophylline, given systemically. (2) It is also possible that drugs administered by aerosol may act predominantly by producing vasoconstriction, thus reducing mucosal congestion and edema, whereas some of the drugs that are effective when given systemically may have additional effects on the pulmonary circulation and bronchiolar smooth muscle. It is of interest that, in this study, aerosolized bronchodilators produced measurable improvement in flow rates in two patients treated with cortisone.

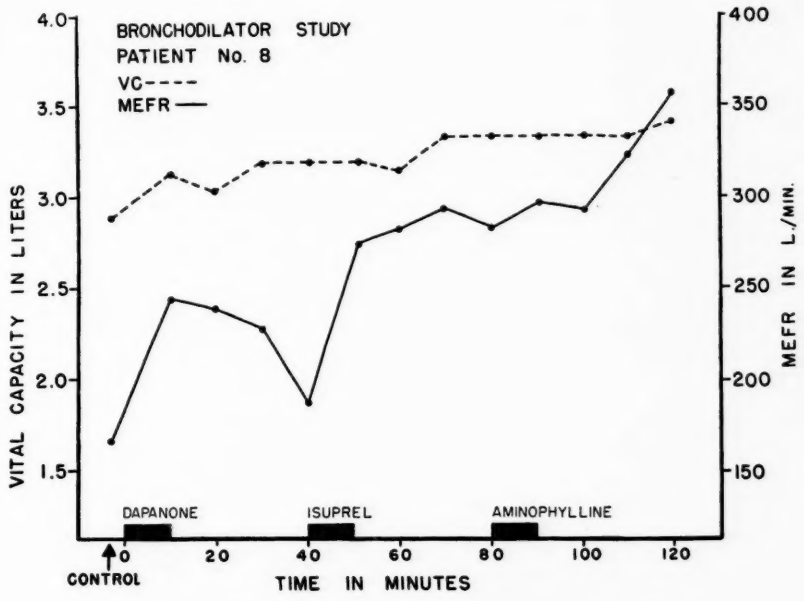

Fig. 2.—Response of Patient 8 to bronchodilators.

The fact that the majority of our patients were unable to achieve normal expiratory flow rates following the bronchodilator therapy employed raises the question as to whether associated conditions such as emphysema, bronchiectasis, or pulmonary vascular sclerosis prevented complete reversibility. However, it is likely that one cannot achieve maximal relief of bronchial obstruction in all patients with the conventional bronchodilators used in this study,[10, 11] and that systemic administration of cortisone is often necessary to produce a more complete return to normal.

The almost complete absence of systemic effects produced by the nebulized bronchodilator drugs demonstrates either that they act only locally or that systemic absorption is insignificant. No systemic effects were noted following the administration of Isuprel, and there was only one complaint (slight, transient headache) following the use of Dapanone. On the other hand, twelve

of the sixteen patients given aminophylline had systemic reactions, consisting of flushing of the face, vertigo, weakness, nervousness, tremors, nausea, and vomiting.

The presence of abnormal pulmonary function, especially the markedly reduced expiratory flow rate, in the interval between asthmatic attacks corroborates the findings of others and again emphasizes the need for more intensive drug therapy during such periods to reduce the incidence of complications in bronchial asthma.[12]

SUMMARY

A simple method for the evaluation of bronchodilator drugs has been used in a group of patients with chronic bronchial obstruction. A new bronchodilator agent, 5 per cent Dapanone, was found to be about as effective as 0.5 per cent Isuprel (both given as aerosols). Maximal benefits, however, were obtained by the simultaneous use of these nebulized drugs and aminophylline, administered intravenously.

Data obtained in this study on the variability in vital capacity and maximal expiratory flow rate in asthmatic patients without therapy reemphasize the need for careful control studies in the evaluation of bronchodilator drugs.

We wish to express our appreciation to Dr. Malcolm Miller for his kind permission to study patients from his clinic and to Mrs. Ruth King and Shirley Latch for their assistance in the early stages of this study.

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THE ROLE OF THE FORMED ELEMENTS OF THE BLOOD IN ALLERGY AND HYPERSENSITIVITY

A Symposium

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INTRODUCTION

DR. SAMTER.—The formed elements of the blood have been stepchildren of allergy for a long time. Until recently, the only cells which were thought to participate in allergic reactions were the eosinophils. Progress during the last decade has been so rapid that it now appears that each of the formed elements of the blood may become involved in immunologic mechanisms.

About ten years ago, Katz exposed red blood cells from ragweed-sensitive patients to ragweed and found that they liberated histamine into the surrounding fluid; even this relatively simple experiment has only now been repeated and reconfirmed. The peculiar delay in our readiness to accept the immunologic potential of white blood cells, red blood cells, and platelets probably is due to our upbringing, which led us to believe that the function of erythrocytes is limited to gas exchange, or of polymorphonuclear leukocytes to phagocytosis. Discussing lymphocytes, Rich in 1936 labeled it "one of the most humiliating and disgraceful gaps in all medical knowledge" that "literally nothing of importance is known of these cells other than that they move and that they reproduce themselves."

It gives me a strange feeling of pride in what has been accomplished to introduce the distinguished panel of guests who will discuss phases of the field in which they have made their contributions: Dr. Sol Haberman, on the red blood cells; Dr. William Dameshek, on the platelets; Dr. Merrill W. Chase, on lymphocytes; Dr. Theodore L. Squier, on polymorphonuclear leukocytes; and Dr. Max Samter, on the eosinophils.

DR. HABERMAN (On Red Blood Cells).—The position of the red cells in allergy is somewhat tenuous at this time. Although erythrocytes rarely have

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been shown to produce clinically discernible hypersensitivity in the strictest sense of the word, they frequently are involved in antigen-antibody reactions that produce harmful results in the body when these occur in vivo. Such reactions are most commonly associated with incompatible transfusion reactions, erythroblastosis, and other neonatal situations.

Until recently there has been very little evidence that the homologous erythrocyte could produce an immunization or hypersensitivity in the same human being. This phenomenon of a person becoming sensitized to his own antigens was called "horror autotoxicus" by Ehrlich and it was thought not to exist. Through the years, the failure to demonstrate clear evidence of such a phenomenon led to the acceptance of the theory of "horror autotoxicus" as an axiom in the science of immunology. However, recent evidence has been found that reopens the question as to the possible occurrence of this immunologic enigma. The demonstration of adsorbed globulin on the surface of erythrocytes of patients suffering from acquired hemolytic anemia by the Coombs antiglobulin test demands a re-examination of this principle

Our knowledge of the antigens of the human red cell has expanded from the four blood groups of Landsteiner and his associates through the M-N antigens of Levine and Landsteiner; the Hr-Rh antigens of Buchbinder, Wiener, and Levine; and at present we recognize and can identify more than thirty well-defined erythrocyte antigens (Table I).

TABLE I. THE KNOWN HUMAN ERYTHROCYTE ANTIGENS

1. ABO (A_1, A_2, A_3, A_4)
2. MNSs
3. Pp
4. CDE-cde (C, C^u, c, c^v, D, D^u, d, E, E^u, e)
5. Ff
6. Kell-Cel
7. Lu^a
8. Le^a, Le^b
9. Fy^a, Fy^b
10. Gr
11. H
12. Jk^a, Jk^b
13. Be
14. Mi
15. "Private" blood antigens

Most of the antigens described today were discovered as a result of isoimmunization due to pregnancy and the increased use of transfusion in therapy. As these antigens are discovered and the means of testing for them are devised, blood transfusions have become safer and consequently the use of transfusions continues to increase. As a result, this has led to more instances of isoimmunization and we are turning up more and more of these new antigens.

The detection of antibodies resulting from isoimmunization to red cells at first followed the classic lines. These approaches were soon found to be inadequate and new techniques were devised for the laboratory demonstration of erythrocyte antibodies. These have included agglutination in saline, use

of bovine albumin and other colloidal suspension media, the blocking test of Wiener (not the "blocking antibody test" with which allergists are familiar) and the Coombs antiglobulin test. The antiglobulin technique is a rediscovery of a method worked out in 1907 by Moreschi, who described a similar test for the detection of weak antibacterial antibodies. Apparently Moreschi was far ahead of his time, and his work had been lost in the literature until Coombs rediscovered the paper after publishing his first report.

By the various methods devised for the demonstration of inapparent and difficult to detect antibodies, attempts have been made to classify the erythrocyte antibody which is produced by isoimmunization. In order to present this problem adequately, it is necessary to describe the attributes of antibodies resulting from such immunizations. The various attempts to describe and designate the antibodies and their attributes have produced a confused nomenclature and a confusion of concepts. Table II presents the three principal attributes or characteristics by which we designate antibodies, namely, their specificity, serologic activity, and chemical qualities. The term *specificity* is

TABLE II. SEROLOGIC AND CHEMICAL CHARACTERIZATION OF SINGLE SPECIFICITY (ANTI-D)

	AGGLUTININ	AGGLUTINOID	CRYPTAGGLUTINOID
Other Terms Used:	Saline agglutinin Complete antibody Heat labile antibody Hydrophilic antibody Early antibody Immune antibody Bivalent antibody	Albumin antibody Incomplete antibody Heat stable antibody Hydrophobic Late antibody Hyperimmune antibody Glutinin Univalent blocking antibody	Albumin antibody Incomplete antibody Heat stable antibody Hydrophobic antibody Late antibody Hyperimmune antibody Glutinin Univalent blocking antibody
Serologic characterization			
Agglutination in saline	+	−	−
Agglutination in albumin	+	−	+
Blocking test	−	+	−
Antiglobulin test	+	+	+
Chemical characterization			
Water-soluble fraction	−	+	+
Salt-soluble fraction			
Alpha	−	±	±
Beta	−	+	+
Gamma	+	−	−
Effect of NiOH			
Antibody adsorbed	−	+	+
Antibody not adsorbed	+	−	+
Precipitation by tannic acid			
Antibody in precipitate	−	+	+
Antibody in supernatant	+	+	+

(From *Am. J. Clin. Path.* **24**: 305-320, 1954, by permission of the editor and the publisher.)

used in the accepted sense, referring to the specificity of the antibody for the antigen (that is, the anti-D reacts only with those erythrocytes containing D). The antibody of that specificity may not give a visible reaction, such as agglutination. The ability to produce certain serologic reactions is determined by its reactivity, as characterized in Table II. The multiplicity of terms used to describe these reactions has led to considerable confusion and is a serious obstacle to successful communication in this field.

In this presentation, I will use three terms to describe the types of serologic reactivity: *agglutinin, agglutinoid,* and *cryptagglutinoid.* One can see from this table that even the agglutinin (originally described by Gruber and Durham in 1896) has been called by a number of terms by workers in the field of immunohematology. The antibody we have designated as the agglutinoid was originally described and so named by Eisenburg and Volk in 1902 for bacterial antibodies which caused prozones. The agglutinoid is capable of specifically adsorbing on the antigen without causing visible clumping. If this antibody globulin is present in sufficient quantity it will block the antigen and prevent subsequent agglutination by agglutinins of the same specificity. These qualities of reactivity have led to the use of such terms as *incomplete antibody, blocking antibody,* etc. The name *cryptagglutinoid* was applied by the present authors to antibody globulins that combine specifically, do not cause agglutination of erythrocytes in saline suspension, and do not give a positive Weiner blocking test but will cause erythrocyte agglutination when 20 per cent bovine albumin is used as the suspension menstruum. Other authors have applied the same terms to this antibody as have been used for the agglutinoid. This application of two sets of terms to three serologic types of antibody reactivity is a source of continuing confusion and ambiguity. Although we have applied the term *cryptagglutinoid* to a serologically distinct type of antibody activity, the chemical characterization indicates that more than one immune globulin may give the serologic reactions used for this differentiation. Chemically, we can show that the cryptagglutinoids occur in several different fractions of the serum proteins (Table VII).

The agglutinin is found in the gamma fraction; the agglutinoid is found mostly in the water-soluble fraction and the salt-soluble beta, with traces in the alpha fraction. The cryptagglutinoid is also found in the water-soluble and beta fractions.

In our hands, certain physicochemical differentiations can be made. Graded amounts of nickelous hydroxide "floc" were found to adsorb the agglutinoids and some, but not all, of the cryptagglutinoids. In view of this and other data, we believe that the cryptagglutinoid is a serologic designation for two types of protein molecules—one found in the salt-soluble beta globulins, the other in the water-soluble fraction. Both of these globulin types give substantially similar serologic reactions.

It is felt that pictorial representation of antigen-antibody reactions often results in dubious concepts. Most of these pictures of antigen-antibody reactions show a "key in the slot" reaction with an implied "mortise and tenon" fixation of antigen to antibody. Although such presentations have

teaching value, they do not present a true picture. Antigens and antibodies react much as other complex organic molecules. The processes of adsorption exhibit examples of mass action, as exemplified in Tables IV and VI.

In the case of agglutinin (Table III), A represents the antigen and B the agglutinin; the mixture of the two results in a combination of antigen and antibody. When two antigens are involved (ABA), the clumping effect is observed. This reaction is reversible. With the agglutinoid, only the AB reaction occurs and there is no agglutination in saline or in albumin suspension. This combination is reversible, but the tendency for adsorption far exceeds the desorption process. In reactions involving the cryptagglutinoid, desorption occurs to a greater extent than with the agglutinoid. Although no agglutination occurs in saline suspension, one can produce clumping by the addition of hydrophilic colloids such as albumin. The albumin does not glue two univalent antibodies together, as has been proposed, but the changes in the physical chemistry of the suspension under these conditions allow the cryptagglutinoid to produce the clumping that did not occur in the presence of saline.

The Coombs test is applicable to the detection of the agglutinoid and cryptagglutinoids. However, it finds little use in the study of the agglutinin. Under ordinary circumstances, the agglutinin may be detected easily and there is little need for this delicate procedure. On the other hand, the non-agglutinating immune globulins (agglutinoids and cryptagglutinoids) demand specialized techniques for their demonstration in the sera of patients.

In the performance of the first stage of the test (Table III), the agglutinin results in ABA combinations giving visible reactions and no further tests need be done. However, the agglutinoid and the cryptagglutinoid fail to cause clumping, with the probable formation of AB combinations which are

TABLE III. ANTIGLOBULIN TEST I
REPRESENTATION OF FIRST-STAGE REACTIONS

$A + B_{[agglutinin]}$	$\rightleftarrows$	ABA	Agglutination
$A + B_{[cryptagglutinoid]}$	$\rightleftarrows$	AB	No agglutination in saline Agglutination in albumin
$A + B_{[agglutinoid]}$	$\rightleftarrows$	AB	No agglutination in saline or albumin

A, Antigen (RBC); B, antibody (e.g., anti-Rh); C, antiglobulin serum; D, normal human globulin.

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not demonstrable as such. The equation shows these combinations as reversible reactions, with the tendency for adsorption dominating the equilibrium. As in other reversible reactions, that equilibrium in favor of dissociation can be effected by the mass action principle. The results of an experiment showing such a dissociation of agglutinating antibody from antigen are presented in Table IV. In this experiment, aliquots of erythrocytes sensitized in serial dilutions of anti-D agglutinins were washed with saline and then observed for agglutination. This process was repeated until the six-

teenth wash yielded no agglutination in any of the dilutions. When these unagglutinated cells were tested by the Coombs reaction no evidence of the originally adsorbed antibodies was found. The antigens of the washed erythrocytes were intact as shown by their ability to react with agglutinins of the anti-D specificity. This experiment is evidence of the reversibility of antigen-antibody complexes, as well as an indication of the number of saline washings that can be tolerated by erythrocyte suspensions.

TABLE IV. EFFECT OF REPEATED WASHING ON AGGLUTINATION OF D+ ERYTHROCYTES BY ANTI-D

NO. OF WASHINGS IN SALINE	DILUTIONS OF ANTI-D							
	1	2	4	8	16	32	64	128
0	++++	++++	++++	+++	+++	++	+	–
1	++++	++++	++++	+++	+++	++	+	–
2	++++	++++	++++	+++	+++	++	+	–
3	++++	++++	++++	++++	+++	++	–	–
4	++++	++++	++++	+++	+++	++	–	–
5	++++	++++	+++	+++	++	–	–	–
6	+++	+++	+++	++	+	–	–	–
7	++++	+++	+++	++	+	–	–	–
8	+++	+++	++	+	–	–	–	–
9	+++	++	++	–	–	–	–	–
10	+++	++	+	–	–	–	–	–
11	+++	++	–	–	–	–	–	–
12	++	+	–	–	–	–	–	–
13	++	–	–	–	–	–	–	–
14	+	–	–	–	–	–	–	–
15	+	–	–	–	–	–	–	–

After sixteenth washing all tubes showing no agglutination gave a negative Coombs test. When tested with anti-D agglutinins, all tubes washed free of agglutinins now gave positive results, showing that D antigen was still present in the cells.

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In the second phase of the Coombs test the relationship of the antiglobulin serum to the sensitizing antibody is important. The animals used for the production of Coombs sera should be immunized with the complete range of human globulins. The sera resulting from such immunization should yield antibodies that react with all the human immune globulins. Theoretically, whole serum or a preparation containing all the globulins is the best antigen. This desirable broad specificity of the Coombs serum cannot be taken for granted unless one makes the unreasonable assumption that the antiglobulin serum is always equally reactive with each type of serum protein in which antibodies may be found. Since antibodies of the Rh and other groups are known to exist in different globulin fractions, it would seem undesirable to attempt to narrow the specificity of Coombs antiglobulin sera by using single globulin fractions in the animal immunization process. It is our feeling that the Coombs test will open up many new phases of study in the broad field of immunology, just as it has done to the narrow Rh field.

Table V represents a continuation of the Coombs reaction after the first stage shows no clumping. The AB combinations having some tendency toward reversibility result in a mixture of AB + A + B + D. The washing of this mixture removes the free B and the D. Some few erythrocytes also are lost

TABLE V. ANTIGLOBULIN TEST II

REPRESENTATION OF SECOND-STAGE REACTIONS
$AB + [A + B + D]$ ↘ lost in washings — Saline washing removes D and some A and B
$AB + A + B + C \left[\begin{array}{c}\text{antiglobulin}\\ \text{agglutinin}\end{array}\right] \rightleftarrows ABCBA + ABCB + ABC + BCB + CBC + BC$ [Agglutination]

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in the process. When the washing process is completed, the washed cells are coated with antibodies and are in a momentary unstable equilibrium with sufficient A for the B present in the tube. At this point in the Coombs test, the antiglobulin serum is added. The antiglobulin, C, is present in high concentration and adsorbs to the B of the AB complexes, giving ABCBA combinations which are visible as clumped erythrocytes. The visible clumping is described as a positive Coombs test.

When the ABCBA complexes are considered, one is forced to conclude that if ABA combinations obey the laws of mass action and form dissociates of ABA, then it should follow that the Coombs complex of ABCBA should do the same. In Table V the possible ABCB + ABC + BCB, etc., are presented as part of the reaction that occurs in this test.

TABLE VI. EFFECT OF TIME OF INCUBATION ON TITRATION BY THE COOMBS TECHNIQUE*

INCUBATION TIME	DILUTIONS OF CRYPTAGGLUTINOID ANTI-D AGAINST CDe/cDE CELLS								
	1	2	4	8	16	32	64	128	256
1 min.	++++	++++	++++	+++	+++	++	++	+	–
5 min.	++++	++++	++++	+++	+++	++	+	±	–
10 min.	++++	++++	++++	+++	+++	++	+	–	–
20 min.	++++	++++	+++	+++	++	+	–	–	–
40 min.	++++	++++	+++	++	+	–	–	–	–
60 min.	++++	+++	+++	+	–	–	–	–	–

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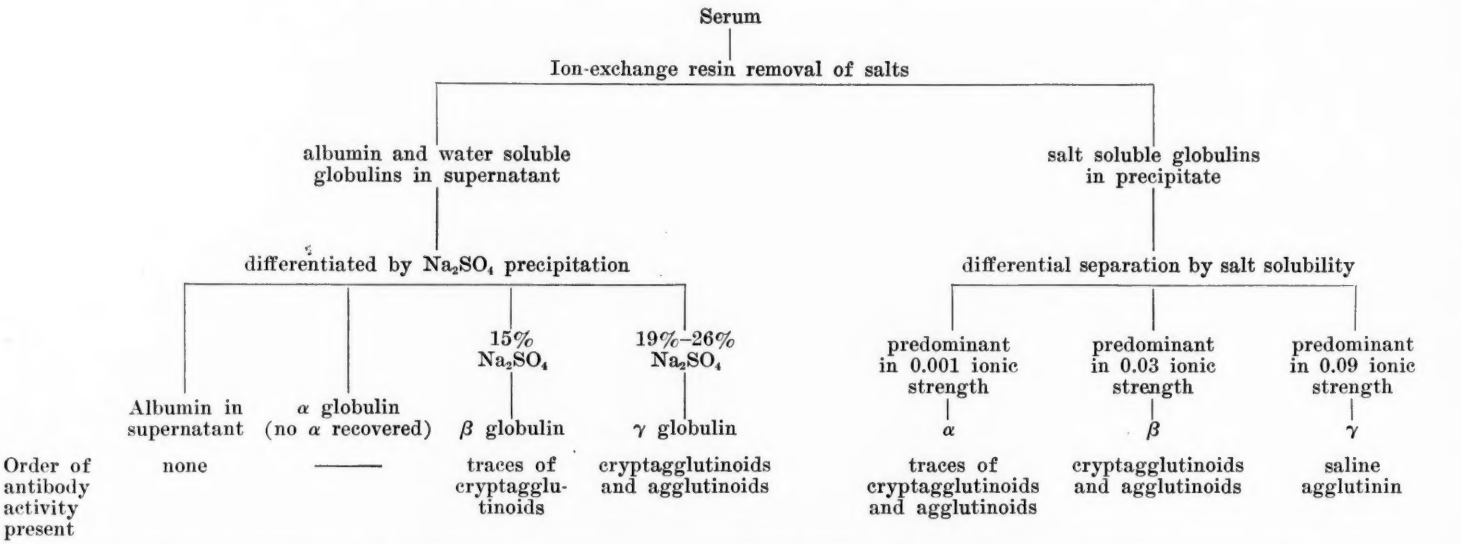
*The anti-D serum had a cryptagglutinoid titer of 1:128 as demonstrated by the indirect Coombs test when the titration was read within 5 minutes. The titer and strength of reaction became weaker as the time of incubation, after the addition of the antiglobulin serum, was prolonged.

Studies on this latter consideration have led to some evidence that such dissociates of the ABCBA complex are formed due to the presence of excesses of C in the potent Coombs sera usually used in this method. An example of such an experiment is presented in Table VI. As time elapses after the addition of potent Coombs antiglobulin serum, the visible agglutination may be observed to weaken. The first tendency after the addition of the Coombs reagent is for the formation of agglutination represented by ABCBA. As time elapses some dissociation to BCB, CBC, CBCBC, etc., occur. The excesses of C favor these complexes at the expense of the agglutinated erythrocytes. The data in Table VI show how such a reaction results in a weakening of agglutination as indicated by the drop in observable titer during prolonged incubation.

TABLE VII



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Schematic representation of anti-Rh serum fractionation by ion-exchange resins and separation of water soluble globulins by Na_2SO_4. (Antibody thus separated identified by salt solubility and/or electrophoresis.)

It should be noted that if the antibodies of the Coombs antiglobulin reagent are of a nonagglutinating variety, prozones or poor reactions will result when it is employed and its usefulness as a diagnostic tool will be destroyed.

When mass action effects on Coombs test complexes (ABCBA) are considered, one might surmise that a very complex combination such as ABCBCBCBA might occur if a realignment of the molecules continues. The electron micrograph presented in Fig. 1 has certain features that are in accord with such a concept. The bridges seen between the two anti-D sensitized erythrocytes, that had been treated by the Coombs method, seem to be made up of ovoid bodies attached end-to-end to form strands between the

Fig. 1.—Electron micrograph showing two erythrocytes, "ghost cells," that have been sensitized with anti-D cryptagglutinoids and agglutinated with Coombs antiglobulin serum. Note the layer of adsorbed material on the red cell membranes and the bridges between cells. Palladium casts. (Magnification, ×65,000, reduced ½.)

(From Finch, Ross, and Ebaugh: *Am. J. Clin. Path.* **24**: 305-320, 1954, by permission of the editor and the publisher.)

cells. The larger bridges might conceivably be composed of multiple strands forming bands of contact between the erythrocytes. The erythrocytes in this picture had been lysed in hypotonic solution so that the erythrocyte "ghost cells" could be photographed. The possibility remains that this picture is fortuitous and represents an artifact.

When the Coombs test is applied to the erythrocytes of patients suffering from acquired hemolytic anemia, some exceedingly interesting data are obtained. When the erythrocytes from such patients are carefully washed in saline and

then mixed with Coombs antiglobulin serum, agglutination occurs. This indicates that these red cells have globulins on their surface which are not present on the surfaces of normal erythrocytes. These findings have been interpreted as evidence of Ehrlich's "horror autotoxicus" and, therefore, these patients are suffering from hemolytic anemia due to the production of antibodies against their own erythrocyte antigens. The proof that such positive Coombs reactions are due to antibody molecules resulting from self-immunization is still forthcoming. The evidence so far presented is convincing and offers the clinician a working hypothesis.

The position of antibody destruction and resulting disease has been proved in the case of erythroblastosis of the newborn and hemolytic transfusion reactions. In these cases the proof of disease production by antibodies is established. In the case of erythroblastosis, the production of antibodies by the mother to antigens in the fetal red cells causes a hemolytic anemia. In the case of ABO hemolytic transfusion reactions, the patient usually has received the wrong blood group and the naturally occurring anti-A or anti-B already present in his plasma destroys the transfused erythrocytes. However, in the Rh-Hr hemolytic transfusion reactions, a different situation exists. The patient has produced the erythrocyte antibody as a result of isoimmunization to previous transfusions or as a result of pregnancy. The antibodies so produced destroy the transfused blood which contains that specific antigen and causes the hemolytic transfusion reaction.

Recently, reports have appeared in relation to the use of ACTH and cortisone in the treatment of erythroblastosis. The results have been most encouraging. The best results have been found when ACTH or cortisone is given to the mother a month or more before delivery of the infant and, on delivery, the child is given an exchange transfusion. In our series, we have included only those mothers who have had at least one affected child and who are married to homozygous Rh positive husbands. The use of ACTH combined with exchange transfusion has resulted in no cases of kernicterus and excellent responses on the part of the affected infants. These findings are in accord with those of Hunter and others.

In the treatment of hemolytic transfusion reactions, the use of ACTH offers encouraging results that are noteworthy. In two cases, patients received the wrong ABO blood group and showed the symptoms of hemolytic transfusion reactions. In both cases, studies on the remains of the administered blood demonstrated that group A blood had been given to group O patients. Within one to three hours after administration ACTH was given intravenously along with compatible blood. Both patients responded quickly and very little evidence of shock occurred. Only a slight degree of hematuria, with no kidney shutdown, resulted. The symptoms were exceedingly mild and neither patient developed sequelae. When antibody studies were made at later dates, definite evidence of antibody titer increases were found. In view of such results, the use of ACTH in the treatment of hemolytic transfusion reaction is worthy of further study.

The results just presented have raised the question in our minds as to the mechanism of ACTH in antigen-antibody reactions. In our studies, we have

found that neither ACTH nor cortisone interferes or affects the in vitro reactions. The agglutination of erythrocytes is unaffected in degree of clumping or titer of reaction by the presence of those substances. Experimental immunization of rabbits, using ACTH and cortisone along with the antigenic stimulus, failed to influence the production of antibodies to human erythrocytes or serum proteins. Both control and test animals gave essentially the same results. However, we have yet to repeat the work reported by Moeschlin, who showed that if ACTH is given early enough before the immunization process and during the stimulation, a depression of antibody production will result. Regardless of the effect of these substances on antibodies and their production, it seems probable that some effect is being exerted by these hormones that protects persons suffering adverse in vivo antigen-antibody reactions, such as erythroblastosis and hemolytic transfusion reactions.

Question: Dr. Haberman, is there any way of distinguishing between agglutinoid and agglutinating antibody by tannic acid adsorption?

Dr. Haberman: We found that certain concentrations of tannic acid seem to have a somewhat elective effect on some of the serum proteins, and we were able to remove them and show that there was a difference in the type of protein removed by this approach. We found that the precipitate resulting from the tannic acid treatment contained the majority of the so-called blocking or agglutinoid antibodies, along with the majority of the cryptagglutinoids.

Since I have the floor, and since Dr. Dameshek is about to follow me, I would like to make one additional comment. Dr. Dameshek and I have been engaging in a friendly running argument about the question of adsorbed antibodies on the red cells of patients having hemolytic anemia. Actually, we are not as far apart as one might think. As early as 1947 we were using the heat elution method of Landsteiner and Miller to remove this adsorbed substance (I still am reluctant to call it a true antibody) from the patients' washed erythrocytes. The resultant eluates were used in the cross-matching and selection of blood for transfusion. This method resulted in successful transfusions and the blood selected by this method seemed to last longer in the patients' blood stream than blood that was not selected by this method. We do not have sufficient cell survival studies to confirm this point, so we must consider it in the light of impression rather than fact.

The point of difference with Dr. Dameshek is on the question of whether or not the erythrocytes of patients having acquired hemolytic anemia are coated with antibodies in the true sense of the word. That is the point we want proved. The fact that it looks like one and seems to act like one does not mean that it is one. You know the old story—"He looks like a duck, he walks like a duck, and he acts like a duck—but he isn't a duck, he's a goose."

DR. DAMESHEK (On the Platelets).—The work in our laboratory on platelet antibodies stems from our previous studies on red cell auto-antibodies. One must distinguish between auto- and iso-hemolytic disease, and one can say that there are *iso-* and *auto-*immunohematologists.

In acquired hemolytic anemia, which had been described a number of years previously by such workers as Chauffard and Widal, Abrami, and Brulé of Paris between 1910 and 1915, we found an auto-hemolysin or an auto-agglutinin present in many of the cases. This resulted in the development of the concept of auto-immunization which has been very fruitful, not only in hemolytic anemia, but in other cytopenic states. In acquired hemolytic anemia, we found a free circulating antibody in the serum of these patients, and later Coombs demonstrated that the antibody was adherent to the surface of the red blood cell. With the availability of ACTH for clinical investigation, we began its use in the attempt to reduce antibody production. This resulted in excellent beneficial effects in most cases.

In idiopathic thrombocytopenic purpura, which has many similarities to acquired hemolytic anemia, we began to think along immunologic lines as well. That the platelet might be antigenic had been known for a number of years. Heteroplatelet immunization with induction of thrombocytopenia had been induced in animals given the platelet antibody. Human isoimmunization was demonstrated more recently in thrombocytopenic patients with hypoplastic anemia who were given repeated transfusions of polycythemic or normal blood. The thrombocytopenia in these cases is associated with a lack of megakaryocytes from the bone marrow; that is, amegakaryocytic thrombocytopenic purpura, quite in contrast with the situation in idiopathic thrombocytopenic purpura (ITP) in which the marrow is crowded with these giant cells.

In patients with amegakaryocytic thrombocytopenia, the administration of transfusions at first resulted in a marked rise in platelet count. With repeated transfusions, the platelet increase became less and less striking, until finally there was practically no rise. This indicated that a factor acting against introduced platelets had developed, and indeed subsequent studies demonstrated that it was a platelet iso-agglutinin. These studies were made possible with the development of new methods of handling platelets and thus preventing their normal agglutinating tendency. Siliconized apparatus, the use of such materials as sequestrene, triton, etc., and the refrigerated centrifuge have all been useful in the development of methods for detecting platelet agglutinins.

Now, for the matter of platelet *auto*-immunization. It was assumed that this might be an important factor, at least in some cases of ITP. Platelet survival studies in these patients indicated a greatly diminished life span, particularly in the acute cases. Whereas in hypoplastic anemia the platelet survival time might be four or five days (at the beginning of transfusion therapy), in chronic ITP it was about one day, and in acute ITP it was a matter of hours only. These studies on platelet survival indicated an extrinsic mechanism which was destroying the platelets within the circulation. Harrington, in St. Louis, performed a more direct experiment demonstrating the same thing. Thus, when he injected blood from patients with ITP into normal persons, the platelet count of the normal was reduced often to extremely low values. This study indicated the presence of a "thrombocytopenic factor," subsequently shown to be a platelet agglutinin. Since this attacked only platelets and was absorbed by platelets, it could be called a platelet antibody, and it fulfilled all the re-

quirements of an immunologic antibody, even though the antigenic factor has never been clearly demonstrated. In one patient (M. K.), there was an extremely large concentration of platelet antibody comprising about 6 per cent of all the serum protein. Studies with this serum, when injected in vivo and by various immunologic, electrophoretic, and chemical methods, indicated that the agglutinin fulfilled all the various requirements of an antibody. Thus, we have been using the term *auto-immune thrombocytopenia* more and more for these cases. An auto-antibody appears to be produced in response to some thus far obscure mechanism which attacks the patient's own platelets and results in marked thrombocytopenia.

The concept of platelet auto-immunization has been bolstered considerably by the work of Ackroyd with Sedormid thrombocytopenia, and by some more recent work on quinidine hypersensitivity. Sedormid, a sedative, was widely used some years ago, particularly in Europe. Its continued or intermittent administration resulted in thrombocytopenia in some patients. Ackroyd showed that this was due to the development of a platelet antibody which was lytic in type and required complement. Our own studies have been concerned with quinidine, which is used rather extensively for various cardiac disorders, notably the arrhythmias. It has been possible to work out the process of auto-immunization in several of these patients. Thus, a patient receiving quinidine in several courses may develop an antibody. This may take some months or, perhaps, years. The platelet antibody that develops does not react with platelets unless quinidine is simultaneously present. The quinidine appears to act as a hapten, which reacts with platelets, perhaps by modifying them, to form an antigen. This quinidine-platelet antigen may then be productive of an antibody, which again is highly specific and reacts only in the presence of quinidine or against quinidized platelets, causing marked agglutination and lysis. In vivo, the patient with quinidine suddenly develops an extreme thrombocytopenia, evidently due to violent platelet destruction in the circulation. Hemorrhagic phenomena develop very quickly, and the patient has a fulminating hemorrhagic purpura, with bleeding from all the various membranes and into the skin. This may occur within an hour or less after a final administration of the quinidine and may be associated with marked leukopenia. The development of the specific drug-platelet reaction is of great importance for various reasons: (a) it is the first time we have been able to demonstrate a definite immunologic abnormality in these "hematoxic reactions" and (b) it is possible that a similar mechanism may be operating in acute thrombocytopenia of the "idiopathic" type. Here, the hapten may be a viral or other protein agent, which can be detected only by special techniques not hitherto developed.

As for the use of ACTH and cortisone in the therapy of thrombocytopenic purpura, our results with those hormones have been by no means as effective as in auto-immune hemolytic anemia. Nevertheless, they are exceedingly useful in the treatment of the acute bleeding crisis of thrombocytopenia, due largely, we believe, to an effect on the blood vessel wall rather than to any considerable increase in platelets.

Dr. Sternberger: Dr. Dameshek, would you comment on your methods of measuring antigen-antibody nitrogen in your patients, particularly in your patient whose antibody titer amounted to 6 per cent of his total serum protein?

Dr. Dameshek: The chemical studies were performed by an expert chemist, Dr. Peter Bernfeld, who performed nitrogen measurements by micro-Kjeldahl apparatus and who carried out the various studies.

Dr. Sternberger: The peculiar nature of platelets raises a number of questions about the nature of such antigen-antibody union. You feel that the antibody accepts complement and reacts with platelets; that in ITP the bone marrow carries an abundance of megakaryocytes; and that the patient, because of so much antibody and so much antigen, must have extensive antigen-antibody reactions. Are these patients depleted of complement?

The use of complement fixation tests in these studies raises another question. While platelets do not carry complete thromboblastin (one of the most anticomplementary substances known), it might theoretically affect the validity of the test. Have you encountered difficulties which might be attributed to the presence of thromboplastin?

Dr. Dameshek: Complement studies were not performed in these cases until recently, when they were carried out by Dr. Fred Bolton. They indicate (a) that there is definite complement consumption during the platelet-antibody reaction and (b) that complement is necessary for platelet lysis, particularly in quinidine thrombocytopenia. This is confirmatory of Ackroyd's work with Sedormid purpura. In cases with high concentration of platelet agglutinin (as in M. K.) complement does not appear to be a factor, the platelets being agglutinated and injured by antibody and then removed, perhaps by the spleen or by some other mechanism.

Question: I should like to ask Dr. Dameshek a question which might not be along the lines of his present interests, although it has to do with platelets. Some years ago Dr. Rocha e Silva attributed an increased output of histamine in anaphylaxis to an explosive destruction of blood platelets and of white blood cells that had become sequestered in the dog, possibly also in rabbit liver. If I recall correctly, he correlated a high histamine content of blood samples obtained immediately after shock with the large number of destroyed platelets. Would you consider Dr. Rocha e Silva's findings as additional evidence for antiplatelet activity in immunologic mechanisms?

Dr. Dameshek: I do not know anything about it, but the platelets contain a great many different chemicals; in fact, we have alluded to the platelets recently as a veritable chemical factory. One definite chemical present in platelets is serotonin. It is possible that histamine is a minor constituent of the blood platelets and that in the presence of a sudden explosive destruction of a large number of platelets, a sudden increase in blood or tissue histamine might take place. If so, this is an obscure phenomenon, and by no means well known.

DR. SQUIER (On Neutrophils).—The sudden disappearance of neutrophils in acute granulocytopenia emphasizes the importance of these cells in immunohematology. Two instances of acute granulocytopenia recently observed exemplify the sudden and often symptomless onset which may occur during chemotherapy. A middle-aged woman seen for routine study of asthma developed typical acute granulocytopenia while under observation. She was taking cortisone and recently had been given propylthiouracil by her physician. The latter drug was stopped, and within a period of five days thereafter granulocyte and total leukocyte numbers returned to normal. The second patient, also a woman, seen because of perennial rhinitis, had no additional complaints. She was taking hydralazine hydrochloride (Apresoline), which had been prescribed approximately seven months before for a relatively minor hypertension. Her initial white count was 3,500 with moderate depression of granulocytes and with normal levels of red cells and platelets. Within a week, the peripheral white blood count was 1,500 with 16 per cent neutrophils and 11 per cent eosinophils. Hydralazine was discontinued and, although she was given cortisone for several weeks, no appreciable change in the differential or white cell count occurred except for disappearance of the relative eosinophilia initially observed. These two patients are representative of clinical problems which confront the clinician when granulocytopenia develops during chemotherapy. The first patient, whose total and granulocyte counts promptly returned to normal levels after the causal drug was stopped, exemplifies the typical course of uncomplicated granulocytopenia due to drug hypersensitivity, such as we described in 1933 in amidopyrine granulocytopenia. Withdrawal of the specific antigenic drug is followed by rapid return of leukocytes to normal levels. The characteristic time interval of approximately four to five days required for recovery is not appreciably altered by medication presumed to stimulate the bone marrow.

Granulocytopenia, such as that observed in the other patient, represents a more difficult etiological problem in which drug hypersensitivity may or may not be primarily concerned. Toxic depression of the bone marrow, aleukemic leukemia, or other unknown factors must be considered as possible causes of the granulocytopenia. No agglutination of leukocytes occurred when an aqueous solution of hydralazine was added to auto-leukocytes suspended in the patient's serum, no lupus erythematosus (L.E.) cells were found, the leukocytes did not appear morphologically abnormal, and the albumin-globulin values were within the normal range. The present discussion will be concerned with considerations that may assist in the understanding of immunohematologic response of the polymorphonuclear leukocytes.

Sturgis and Bethel name four basic mechanisms responsible for changes in the numbers of circulating white blood cells: (1) aberrations in the rate of production, either from inhibition or maturation arrest in the bone marrow or from simple hyperplasia of bone marrow or accelerated maturation; (2) changes in the relative number of cells due to differences in concentration of the circulating blood plasma; (3) changes caused by redistribution and sequestration of cells in vascular channels or organs; and (4) alterations in the rate of destruction or elimination of leukocytes from the body. Rapid changes in the numbers

of circulating leukocytes can occur, therefore, from varied causes, either singly or in combination. Redistribution and sequestration of leukocytes can occur from treatment with various drugs, simple shock, exposure to cold, or other alarming stimuli including anaphylactic shock, in which increased leukocyte destruction may occur in addition. It has been mentioned previously that blood responses are never static and that from moment to moment and from hour to hour there is constant change, so that leukopenia observed at one moment may be supplanted in the next by leukocytosis. These facts make clinical interpretation of the significance of any momentary given change in the number or differential type of circulating leukocytes uncertain and difficult. Transitory leukopenia of shock, whether specific or nonspecific, may be succeeded by release of leukocytes sequestered in vessels or organs and resultant increase in circulating numbers. Clumping of leukocytes, whether due to specific leukocyte agglutinins or to nonspecific causes such as Fleck described as "leukergie," may result in prompt leukopenia. This may be followed at times by increased destruction of clumped or damaged leukocytes leading to more prolonged depression in leukocyte numbers.

The circulatory changes after anaphylactic shock have been described by Abel and Schenck. They observed, successively, contraction of arterioles, slowing of the circulation, and finally increased adherence of leukocytes with formation of embolic clumps blocking the circulation of many capillaries and venules in the ears of rabbits during anaphylactic shock. Reduced peripheral blood flow, together with loose attachment of leukocytes to the vessel walls, necessarily resulted in a reduction of the peripheral leukocyte count. Transient removal of leukocytes within the pulmonary circulation is believed by Bierman to account for the marked leukopenia he observed after intravenous injection of histamine. Counts made from blood samples of the catheterized right ventricle and auricle indicated that pulmonary sequestration of leukocytes occurred which primarily involved granulocytes. It has been observed that transfused leukocytes are removed from the circulation promptly or are destroyed. Weisberger transfused rabbits with actively ameboid and phagocytic leukocytes obtained from peritoneal exudates of the same animal. Sudden profound leukopenia, which persisted for about two hours, occurred in most transfused animals. In more than one-half of these rabbits the initial leukopenia was followed by leukocytosis. A similar leukopenia followed intravenous injection of supernatant fluid obtained by centrifugation of auto-leukocytes disintegrated by supersonic virbration. Although Weisberger also observed a mild and delayed leukopenia in three of ten rabbits injected intravenously with histamine phosphate, he did not consider histamine release to be the cause of the leukopenia after leukocyte transfusions. Neither the time of onset nor the degree of leukopenia produced was comparable and, furthermore, the leukopenia after leukocyte transfusion was not prevented by intravenous administration of an antihistamine (pyranisamine maleate). Accordingly, he believed that some substance in the transfused leukocytes caused not only their prompt disappearance, but the disappearance of the white cells already present in the circulation as well. Similar transfusion experiments, in which the leukocytes were labeled with radioactive phosphorus, led to marked

accumulation of radioactivity in the lungs. Histologic sections taken after such leukocyte transfusion experiments showed the interalveolar septa to be engorged with granulocytes. In addition to evidence for sequestration and destruction of leukocytes in the lung, the presence of radioactivity in the liver and spleen after transfusion of labeled leukocytes indicated that these organs participate in leukocyte removal in a lesser degree. The important role of the lung in the removal of transfused leukocytes (and, by inference, of agglutinated or disintegrating leukocytes) is evident, and Weisberger suggested that the lung may well serve as a homeostatic mechanism in the maintenance of normal leukocyte levels.

Nonspecific destruction of leukocytes by lytic factors has been demonstrated, at least in vitro. In 1906, Hektoen observed destruction of leukocytes after contact with a relatively heat-stable streptococcal filtrate, called "leukocidin." Dennis and Senekjian observed in vitro lysis, essentially limited to granulocytes, when typhoid filtrate was added to normal bloods in dilutions as high as 1:1,000. This thermostable lytic substance was quantitatively inhibited by specific immune globulin. However, leukocytes from normal bloods were lysed as readily as those from typhoid patients, so the lysis could not be attributed to an antigen-antibody reaction.

Most attempts to demonstrate specific lysis of leukocytes have given equivocal results. During horse serum anaphylaxis in dogs, Webb found leukopenia in capillary blood from the liver, intestines, and kidney which was entirely comparable to that in the peripheral circulation. He found appreciably fewer leukocytes in blood from the pulmonary vein than from the pulmonary artery and demonstrated sequestration of leukocytes in the lung during anaphylactic shock. However, he was unable to demonstrate destruction of leukocytes, either in vitro or in vivo, in the blood of sensitized dogs when horse serum was used as the antigen. Squier and Lee reported in vitro lysis of leukocytes after the addition of ragweed antigen to bloods from untreated ragweed-sensitive patients. Franklin and Lowell were unable to duplicate these results and it is possible that nonspecific factors alone were responsible for the leukocytolysis observed. The original experiments were made with ragweed antigen with a pH of approximately 4.6, and subsequent experiments in which the pH was adjusted to 7.4 failed to cause significant leukocyte lysis. During the past year Byron Waksman has reported specific leukocytolysis after the addition of homologous antigen to fresh heparinized bloods of rabbits immunized with crystalline hen ovalbumin, or with bovine globulin (Armour Fraction II). Dilutions of the antisera were found to reduce the amount of antigen needed for maximum lysis, and an optimum concentration or range of concentrations of antigen was found which would cause maximum lysis when the amount of antibody was kept constant. Waksman concluded from his experiments that the leukocytolytic factor was associated with a nonprecipitable antibody. There appeared to be no relation between the specific lytic capacity of any antiserum and its content of precipitable antibody N., and the antibody was well correlated with ability to induce urticarial whealing reactions in the human skin or in the skin of sen-

sitized guinea pigs, although heating at 55 to 58° C, for four hours failed to destroy the lytic capacity of three of four antisera tested.

Experiments by Walzer and Israel suggest that antibody of the reagin type may be carried or fixed by leukocytes. Cutaneous sites in five normal recipients were injected with leukocytes from ragweed-sensitive patients after the cells were prepared by washing four times in Tyrode's solution. In every instance these sites responded with positive reactions when subsequently tested with ragweed antigen. If all serum containing ragweed reagin were removed from the leukocytes used, the results would indicate that the sensitizing antibody must have been carried by the leukocytes.

Immunologic lymphocytolysis has been adequately demonstrated in bacterial sensitivities of the tuberculin type. Favour demonstrated lymphocytolysis after addition of tuberculoprotein to suspensions of tuberculous guinea pig cells which were resuspended after clumped granulocytes had largely settled out of the buffy coat. Lymphocytolysis was followed later by destruction of granulocytes and monocytes, as well as of some of the remaining small, mature lymphocytes not previously lysed. Favour regarded this as a disease-specific cytotoxic phenomenon, but did not consider it to be a manifestation of anaphylaxis. Subsequently, Fremont-Smith and Favour, using suspensions in human serum of leukocytes from patients with active tuberculosis, demonstrated lysis in such cell suspensions after incubation following addition of tuberculin. Cytolysis was not limited to lymphocytes, since 20 to 90 per cent of the leukocytes, as well as 20 to 60 per cent of lymphocytes, in bloods from patients with active tuberculosis were specifically destroyed in vitro after contact with tuberculin. Complement appeared essential, and no cytolysis occurred when heat-inactivated serum (56° C. for fifteen minutes) was substituted for fresh normal serum in the experiments.

Immunologic agglutination of leukocytes may be an important cause of certain leukopenias. However, the presence of specific agglutination is difficult to demonstrate satisfactorily. Nonspecific agglutination, such as that described by Fleck and termed "leukergy," may be undistinguishable from immunologic agglutination. In the presence of "leukergy" a drop of citrated blood on a cover glass, dried after agitation at room temperature and stained with methylene blue, showed loose clumps of neutrophil cells and compact clumps of lymphocytes. Masses of pure neutrophils, of pure lymphocytes, of pure platelets, and also masses containing both leukocytes and platelets were observed in such preparations. Fleck found that the addition of heparin to normal blood caused agglutination of thrombocytes and leukocytes at the end of one-half hour and there was seen the same tendency to homogeneity of clumps as in "leukergy." Fleck found leukocyte agglutination and the specific agglutination of rabbit or guinea pig leukocytes by antileukocytic sera no different from that observed in "leukergy."

Dogs treated with guinea pig leukocyte antigen by Witebsky and Komiya developed antibodies against an alcoholic guinea pig leukocyte extract in addition to heterogenic Forssman antibodies. However, since the leukocytes used showed a tendency to auto-agglutination ("leukergy"), an unequivocal agglutinative influence of the antiserum could not be determined, nor was there any

microscopically recognizable lysis of leukocytes after the addition of complement. In vivo experiments by Chew, Stevens, and Lawrence did demonstrate a striking and apparently specific destruction of guinea pig leukocytes. They prepared guinea pig leukocyte antiserum by immunizing rabbits with guinea pig leukocytes. The resulting antiserum was absorbed repeatedly with washed guinea pig erythrocytes, and inactivated to destroy complement without any observed decrease in antileukocytic power. Intracardial injection of guinea pigs with this antileukocytic serum caused disappearance of neutrophils from the circulating blood within five minutes. It was evident, furthermore, that leukocyte disintegration after antibody contact was rapid, since recognizable leukocytes could not be found either in the circulating blood or later sequestered in tissues. A similar, but delayed, disappearance of leukocytes from the circulation was observed within seven hours after intraperitoneal injection of the leukocyte antiserum. Neutropenia continued for twenty-four to forty-eight hours, and then seventy-two to ninety-six hours later the circulating neutrophils returned to normal or somewhat increased numbers. Red cells and platelets were not significantly altered in these experiments and eosinophils, basophils, and monocytes reacted much as they did to similar injections of normal rabbit serum. Although tolerance to the antileukocytic serum appeared to develop after repeated injections of equal doses, induced neutropenia could be prolonged to four or five days if increasing amounts of antiserum were injected each day. Degenerating neutrophils, characterized by swollen nuclei, amorphous appearing chromatin, basophilia of cytoplasm, and irregularity of cell outline, were found occasionally in the circulating leukocytes after intracardiac injections of the antileukocyte serum. Myeloid hyperplasia occurred in the bone marrows of repeatedly injected animals, and differential counts of marrow cells during the leukopenic reaction showed that the rate of decrease of adult neutrophils was slow as compared with the disappearance of neutrophils from the circulating blood.

Definite granulocytopenia lasting from three to five hours was induced in two different compatible recipients by Moeschlin and Wagner. These human transfusion experiments were similar to those of Sprague, Harrington and others, in which transfusion of antithrombocytic human blood induced thrombocytopenia. At the time induced granulocytopenia was maximal; three hours after amidopyrine had been readministered to the sensitive patient, blood was withdrawn and transfused into normal experimental subjects. In each instance granulocytopenia followed. Control transfusions of blood taken from normal subjects after similar ingestion of amidopyrine resulted in no decrease in leukocytes. In addition, the presence of a factor which caused agglutination of homologous and heterologous leukocytes was demonstrated by in vitro experiments with plasma and serum taken from the patient at the height of the induced amidopyrine granulocytopenia. Although a constant and striking increased agglutination occurred, spontaneous lysis of granulocytes did not appear to be increased by the addition of the patient's plasma or serum, even though it was taken at the height of the leukopenic reaction.

Significant contributions to knowledge of the immunologic behavior of leukocytes has been made by Finch and his associates. Leukocytes from patients with chronic granulocytic and chronic lymphocytic leukemias were suspended in Freund's adjuvant and injected into rabbits to produce experimental antileukocytic and antilymphocytic sera. Antisera were absorbed three times with equal volumes of saline-washed type AB and type O human erythrocytes. Three striking morphologic features were observed in stained smears of human leukocytes after incubation with leukocyte antiserum, and described as follows: (1) There was clumping of leukocytes into clusters which frequently resembled the "rosettes" of the L.E. test (Fig. 2). (2) There was phagocytosis of granulocytes. Most of the phagocytic cells contained an intact neutrophil granulocyte,

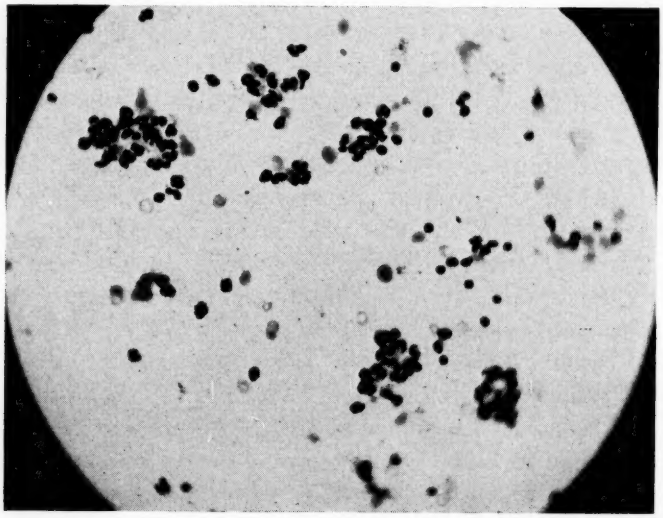

Fig. 2.—Clumping of normal polymorphonuclear leukocytes following incubation with antigranulocytic serum.

(From Finch, Ross, and Ebaugh: *Am. J. Clin. Path.* 24: 305-320, 1954, by permission of the editor and the publisher.)

which was surrounded by the deep blue nucleus and a rim of light blue protoplasm (Fig. 3); a few contained an eosinophil granulocyte. Some cells contained a deep purplish blue cytoplasmic inclusion with appearance indistinguishable from Hargrave's L.E. type cells. (3) There was evidence of lytic leukocyte changes in that most of the nonphagocytized neutrophilic granulocytes developed multiple cystic vacuoles, but the lymphocytes appeared morphologically intact (Fig. 4). Studies, by either sedimentation or tube agglutination, demonstrated that antigranulocytic serum produced marked agglutination of human granulocytes, but not of lymphocytes. Antilymphocytic serum appeared to have very limited agglutinative capacity for either granulocytes or lymphocytes. Absorption of antigranulocytic or antilymphocytic serum with saline-washed human granulocytes reduced or removed the capacity to agglutinate leukocytes, but similar absorption of either serum with saline-washed human lymphocytes was without effect. Leukophagocytic activity was reduced by heating the anti-

leukocytic serum to 56° C. for thirty minutes. The authors reported that by no method were they able to demonstrate complete dissolution of leukocytes after exposure to antileukocytic serum. They believed a specific human antileukocyte antibody present in the rabbit serum to be the most likely cause of the leukophagocytosis and agglutination observed. During in vitro incubation,

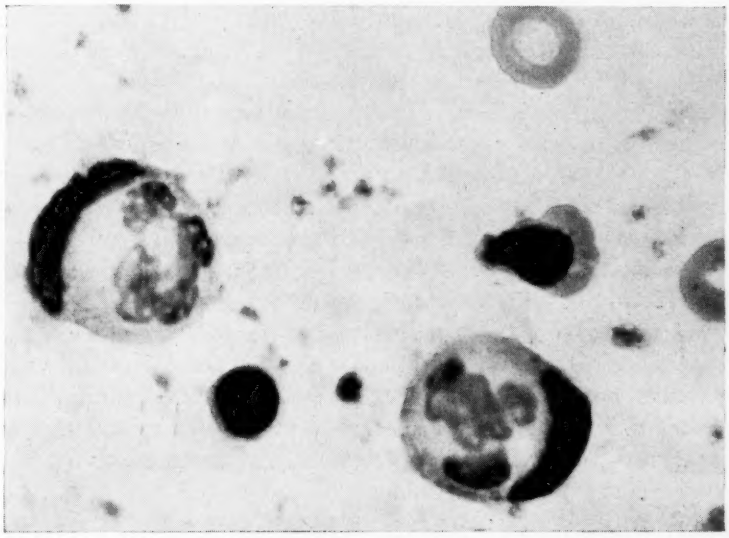

Fig. 3.—Two polymorphonuclear leukocytes, each completely engulfed by a monocyte. (From Finch, Ross, and Ebaum: *Am. J. Clin. Path.* 24: 305-320, 1954, by permission of the editor and the publisher.)

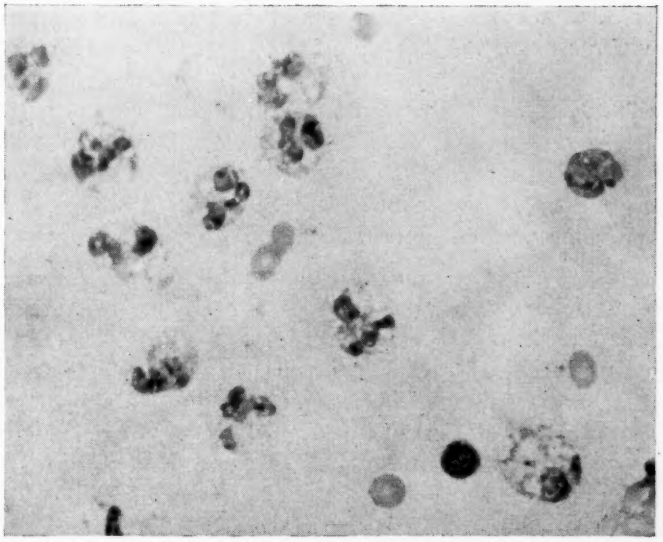

Fig. 4.—Multiple cytoplasmic vacuoles, distortion of all outlines, and nuclear degeneration of polymorphonuclear following incubation with antigranulocytic serum.

(From Finch, Ross, and Ebaum: *Am. J. Clin. Path.* 24: 305-320, 1954, by permission of the editor and the publisher.)

it is believed that the antileukocytic antibody rapidly coats leukocytes, and in the presence of complement, alterations in the cell membrane occur, after which leukocytes are phagocytized and clumped. Sufficient similarity exists between this hetero-immune leukocyte antibody and the L.E. factor to lead Finch and his associates to believe it closely related to or identical with the L.E. factor. However, they found that the antileukocytic antibody induced greater phagocytic activity by monocytes as compared with the increased phagocytic activity by neutrophils associated with the L.E. factor. Also, in leukocyte antisera granulocytes were ingested intact and then destroyed, in contrast to L.E. sera in which neucleolysis of leukocytes is believed to precede phagocytosis of nuclear debris. These experiments indicate that leukocytic antisera can induce the formation of cells closely resembling those caused by the L.E. factor. Accordingly, it is not surprising that L.E. cells have been observed during hypersensitivity reactions as in each of three patients with severe penicillin reactions in whom L.E. cells were found in the bone marrow by Walsh and Zimmerman.

Untoward reactions to hydralazine were observed by Dustan and her associates in thirteen of 139 patients under prolonged treatment. In five of those with more severe manifestations a febrile phase ensued, which resembled acute lupus erythematosus. In one patient of this group there was a positive plasma L.E. test, and in another L.E. cells and rosettes were found in the bone marrow. One additional patient, who had been receiving hydralazine and who had no symptoms, was found to have a positive plasma L.E. test. The phagocytic activity of leukocytes under the stimulus of leukocyte antisera described by Finch strongly suggests that the presumed L.E. cells observed in some of these hydralazine-intolerant patients may be tangible evidence of the presence of leukocytic antibodies and of drug hypersensitivity.

Comment: I would like to add a remark to Dr. Squier's report that it is impossible to increase the number of circulating leukocytes in the rabbit by infusing cells collected from the peritoneal cavity after aleuronite irritation. Such added cells disappear. Dr. Stetson studied this phenomenon and found that cells which had undergone diapedesis behaved differently—their inability to maintain themselves in the blood stream is one of the differences. He concludes that cells in exudates are changed cells, a fact which might become relevant in interpreting the meaning of sequestration.

Question: If one prepares an anti-human leukocyte serum (using the rabbit as the intermediary, as was done by Finch) one can demonstrate the formation of rosettes, which Dr. Squier referred to and which represents, or at least resembles, a positive L.E. test. However, in previous attempts to produce an antileukocyte serum this particular manifestation does not seem to have occurred. Several forms of leukopenia, I believe, are associated with lysis, perhaps of the white cell series without the appearance of a positive L.E. test. Is there any difference, therefore, between the serum or a system which produces lysis or leukopenia, if it is or if it is not associated with an L.E. phenomenon? In other words, does a negative L.E. test suggest that an immune mechanism is absent, and a positive test that an immune mechanism is involved in the mechanism of the leukopenia?

Dr. Squier: I cannot answer the question. I do not know, but will "pass the ball," so to speak, and wonder whether the relationship of antibody and the amount of complement might not play a determining role in the phenomenon. Apparently in some instances, surprisingly enough, inadequate complement intereferes with the demonstration of the leukocyte-agglutinating phenomenon. In experiments with leukocytolysis attempted by us, which apparently failed, complement may have been inadequate. Failure to determine the presence or absence of adequate complement in each test makes repetition necessary. I think it is possible that the presence or absence of adequate complement may be the determining factor.

Dr. Dameshek: I was hoping someone would bring up the question of the L.E. phenomenon, because that is a very exciting development about which we still have much to learn. I believe that most of the evidence indicates that the L.E. phenomenon is immunologic in nature. Is it specific for disseminated lupus? In large measure it seems to be, but we wonder whether the development of the L.E. phenomenon in certain hypersensitivity states indicates that these are formes frustes of disseminated lupus or whether the phenomenon is nonspecific. Apresoline hypersensitivity or "toxicity" with the development of arthritis, hyperglobulinemia, and the L.E. phenomenon is a case in point. Is this L.E., or is it an immunologic phenomenon with the nonspecific development of the L.E. factor? A forthright answer to this question cannot be given, but it is possible to tie up the two disturbances, and say that disseminated lupus is an immunologic disorder brought about by unknown factors whereas Apresoline hypersensitivity with the L.E. phenomenon is also an immunologic disorder brought about by a specific chemical. It is possible that the chemical may act like quinidine and Sedormid to serve as a hapten with such tissues as the vascular endothelium, the white cells. It is probable that many chemical hypersensitivities act through an indirect or immunologic mechanism, which eventually leads to disorders involving the red cells, white cells, platelets, the entire marrow, the blood vessel wall, or the skin. In what way does the L.E. factor differ from a white cell agglutinating antibody? We can say that the two are entirely different and that one may exist without the other being present. It is possible that the L.E. factor in the plasma contains an opsonizing substance preparing certain white cells, notably granulocytes, for the phagocytosis of other cellular material. On the other hand, in the leukocytic heteroimmunization experiments of Stewart Finch, there is no question that peculiar rosette manifestations and phagocytic inclusions take place closely resembling the L.E. phenomenon. This may be the same as the "tart cell" phenomenon of Dr. Hargraves of the Mayo Clinic. I think we can say that the production of leukocytic agglutinin is an immunologic phenomenon, as is the L.E. phenomenon, but that they differ to some extent as to various types of red cell agglutinins and hemolysins.

Dr. Squier: May I say one or two words more, particularly in relation to the "Apresoline phenomenon," which is a very fascinating one to me? There is no evidence at present that the patient I referred to does, in fact, have an immunologic cause for her granulocytopenia. I believe she does, but I have not

been able to demonstrate it. On the other hand, her granulocytopenia may be purely toxic. It is interesting that her white count, when we first saw her, was 3,500 and that she had no complaint except rhinitis. She did not have any of the rheumatic symptoms such as those described in some cases of Apresoline intolerance. When she was next seen a week later, her white count was 1,500, where it has remained almost constant until now (about two months). She did not have any disturbance in blood protein; there was no albumin-globulin reversal. There was no demonstrable agglutination and there was no significant change in the appearance of her leukocytes after we added a solution of Apresoline to her blood. Nor was there any difference in the quantitative complement of her blood as compared with that in normal persons.

DR. CHASE (On Lymphocytes).—The topic of lymphocytes in hypersensitivity, which owes its origin to studies made initially with guinea pigs and afterward with rabbits, is now under study in man. In other words, this field of exploration has arisen by reason of animal experimentation, rather than by primary study of the human being; in its basic form, it deals with the role of lymphocytes in hypersensitivities of the contact dermatitis type and in bacterial allergies. Close assessment of the lymphocyte as the effective cell in suspensions of white cells is indeed relatively recent. Your familiarity with the difficulty of separating varieties of white cells will make it obvious how important it is that one or two workers appear to have conducted their experiments with suspensions that consisted of 95 per cent or more of viable lymphocytes. In the time at our disposal, we can touch only briefly on the major aspects of current studies.

Some years ago, in the laboratory of the late Karl Landsteiner, experiments were in progress on the sensitization of guinea pigs by means of allergenic simple chemicals. In the course of many trials aimed at the transfer of contact dermatitis of the guinea pig to normal guinea pigs, the phenomenon of the role played by the white cells was unwittingly hit upon. Cell-rich exudates caused to appear within the peritoneal cavity of sensitized guinea pigs constituted a source of formed cellular elements that, washed free of adhering tissue fluids and injected into a normal guinea pig, would impose upon the normal animal the specific hypersensitivity that the donor had possessed. No such result could be attained either with serum or with the clarified exudative fluid itself. Transfers of this sort have been elaborated and studied now over many years. They can be done with predictable outcome. Sensitization has been transferred by means of cells in a variety of allergies to drugs (picryl chloride, 2:4 dinitrochlorobenzene, orthochlorobenzoyl chloride and the like); all these are concerned with contact reactions of the delayed type. Your printed program carries a report of the cellular transfer of hypersensitivity to Salvarsan in the guinea pig. This instance, instead of being simply an addition to the list of successful transfers, should be of help in deciding the relative roles played by cells and serum-borne antibodies in the rather special sort of reaction that is elicited by Salvarsan.

The next development was the demonstration that tuberculin hypersensitivity can be transferred to normal individuals by means of the cells taken from tuberculous guinea pigs or guinea pigs rendered sensitive to tuberculin by injection of killed tubercle bacilli. Beyond the facts that we contributed, Drs.

Kirchheimer and Weiser showed that cellular transfer from the tuberculous animal imposes upon the recipient also a capacity for undergoing *systemic* reactions to tuberculin. For the first time, one had evidence of a common mechanism operating in ostensibly different areas of allergy, namely, bacterial allergy and the guinea pig's contact dermatitis. A second instance of bacterial hypersensitivity, Brucella sensitization of the guinea pig, was shown in France to be transferred likewise with cells.

In the first work, as well as in later studies, the success of the transfer was found to depend upon the use of fresh, viable cells. If the cells were exposed to chloroform-saturated isotonic fluid, to heating at 46 to 48 degrees for a few minutes, or to freezings and thawings, all procedures that lead to death of the cells as evidenced by the penetration and staining of their nuclei by trypan blue, then sensitivity would not develop after transfer of the cells in their menstruum. Nor could we make rabbits sensitive to tuberculin by means of cells removed from sensitive guinea pigs, or vice versa. Sensitivity arose in the recipient in from twelve hours to two and one-half days, depending upon the route of administration of the cells. The necessary volume of cells was proportional to the degree of sensitization possessed by the donors.

The sources of cells most readily available yield mixtures of cell types, that is, peritoneal exudates (preferably induced by paraffin oil, rather than by direct irritants that yield predominantly polymorphonuclear leukocytes), peripheral blood affording white cells through the use of isolation techniques, cells recovered from minced spleens or lungs. In our earliest experiments done with mixed types of cells, success in effecting transfer of dermal hypersensitivity appeared to be predictable when the proportion of lymphocytes was high. As a further important step, Haxthausen, in Denmark, the first to confirm the principle of cellular transfer in drug allergy, successfully transferred hypersensitivity to 2:4 dinitrochlorobenzene by means of cells teased from the thymus gland of guinea pigs; he considered that he had used only viable lymphocytes. Another advance was made by Wesslén, in Sweden, who laboriously collected lymph from the thoracic duct of guinea pigs and effected transfer of tuberculin hypersensitivity by the washed cellular elements, said to be at least 99 per cent lymphocytes. As a less precise, but technically more feasible, source of cells that are predominantly lymphocytes, we have long employed whole lymph nodes. When these are dissected out and teased under fluid, lymphocytes streamed out of the ruptured sinuses. Indeed, apart from lymphocytes, the whole complex of cells that are seen upon sectioning intact lymph nodes remains mostly attached to the stroma of the organ and is discarded with it. Some trauma is incurred, and examination with trypan blue reveals that perhaps 20 per cent of the cells are not living; of the remainder, about 90 to 95 per cent are identified as adult lymphocytes. We have not found plasma cells among the few cells that are not lymphocytes. (It should be stated that when analogous teasings are made of stimulated rabbit popliteal nodes, the cellular variety is greater than we have noted in our work with the guinea pig; the cells from rabbit nodes have been studied in considerable detail by Dr. Rebuck.) Our studies indicate that, in all probability, polymorphonuclear leukocytes and erythrocytes can be

excluded as "transferring agents." It is not known whether monocytes or plasma cells, could they be secured in quantity and employed as isolated cells, would play their own roles in transferring dermal hypersensitivity; an active role for the monocytes was not suggested in some early observations made with exudates containing monocytes in rather large proportion and lymphocytes in low numbers.

The demonstrated relationship of cells to hypersensitivity in animals has prompted the making of studies with human white cells. We have seen in the foregoing the basis for centering attention on the lymphocyte, and we sense a new awareness of the presence of local accumulations of lymphocytes at the sites of specific dermal reactions in man. Transfer experiments in man have been undertaken with white cells from the peripheral blood. Usually 50 c.ml. portions of blood have been withdrawn from sensitive persons, the red cells caused to sediment by addition of Fibrogen, and the remaining white cells separated and used for transfer by the intracutaneous or subcutaneous routes of injection. With this technique, Dr. Lawrence of New York University has obtained acceptable and consistent evidence of transfer of tuberculin hypersensitivity to previously nonreactive persons and, in parallel experiments, he has demonstrated transfer of delayed type hypersensitivity to various streptococcal fractions. Indeed, as might have been anticipated from the greater degree of dermal hypersensitivity exhibited by human beings, the potentiality for sensitization by means of transferred white cells seems to be greater than in the guinea pig. It so happens that Dr. Lawrence's technical procedure of separating the cells causes a marked loss of polymorphonuclear cells, for the final suspensions are electively rich in lymphocytes and do not represent the cellular ratios seen in peripheral blood. The duration of transferred hypersensitivity has been some months, at least, although the data should be analyzed for the possible occurrence of two processes that could be revealed as a bimodal curve, one being a state of sensitivity developing shortly after transfer and another being a state of sensitivity of secondary origin.

The technique of cell transfer has been attempted by Drs. Sulzberger and Baer, who selected as donors persons exhibiting drug allergies of the contact dermatitis type. A few recipient patients became sensitive, and in these instances the newly appearing allergy has persisted for long, as in Dr. Lawrence's cases. Consequently, Dr. Sulzberger has attributed his results to an active sensitization induced by material provided by the cells themselves, although it would seem difficult to exclude an influence of the skin tests made in connection with the procedure. One can hardly believe that more will not be forthcoming on the transfer of contact dermatitis from man to man by means of cells.

We may ask, then, whether there exists any clue as to a mechanism underlying the transfer of delayed type hypersensitivity by means of cell suspensions. Such a clue was sought for and finally found, again in the guinea pig. We are not yet in a position to assess it or to judge if it is, with respect to delayed type hypersensitivity, a false clue. As an immunologic observation, it seems established adequately, for it was discovered that the procedure of transferring cells from sensitized guinea pigs to normal ones causes circulating antibody to ap-

pear in the recipient animal in minute amount, the antibody being, like the antibody that is present in the donors themselves and exists in parallel with the state of delayed type hypersensitivity, related to the chemical employed in establishing the donors' sensitization. Careful search has failed to reveal within the cells any appreciable part of the antibody that appears in the serum of the recipients. The result does not depend upon the coexistence of a dermal contact type hypersensitivity; hence, guinea pigs and rabbits that have been immunized and are producing antibody can provide cells that are effective in the "transfer" of antibody production. Several types of antibody have now been detected in recipient animals: guinea pig antibody to picrylated proteins, guinea pig and rabbit diphtheric antitoxin, and (Harris and Harris) rabbit antibacterial agglutinating antibodies. The newly arising antibody accumulates gradually in the recipient animal over the course of a few days and then characteristically declines; the one exception to this is that a second rise in antibody will be found in certain cases in which the cells are removed from donors at a time when frankly antigenic material will be transferred with them. Even if this occurs, however, either peak of such a bimodal curve of antibody concentrations can be isolated by technical means, so that there need be no confusion.

The same sources of cells (spleens or lymph nodes or peripheral lymph), the same requirement for viability of the cells, the same transient course of appearance, and the same requirement that transfer be made within the experimental species appear to apply as in the transfer of dermal hypersensitivity. (These conclusions, of course, may require emendation in the light of further work; Wesslén claims to have crossed the "species barrier.") The antibody that comes into the serum of the recipient animal after injection of cells has been said by Dr. T. N. Harris to be something like 350 times as much in quantity as has been extracted directly from lymph node cells by Dr. Harris' previous techniques. The amount of antibody is, nevertheless, quite small in terms of quantitative immunochemistry. To determine the actual amount as precisely as possible, Dr. Odd A. Wager and I selected diphtheria antitoxin as an antibody that could be evaluated at very high dilution and yet to within 10 per cent or less of its actual concentration. Our figures are based on actual, proved neutralization of diphtheria toxin, with the "antitoxic units" transposed to milligrams of antibody-nitrogen by means of the factor, 2.6. In these studies, the total amount of antibody-nitrogen present in serum following rabbit-to-rabbit transfer was typically 80 to 125 μg following the injection of about 1.5 ml. cells, and about 40 to 60 μg in guinea pig-to-guinea pig transfer using about 0.6 ml. cells, although in one instance there was no less than 500 μg. To complete the picture, the figures given must be multiplied by a factor of about 2.4 to account for the antibody that penetrates into the tissues and is not retained in the blood stream; still another correction may be necessary when the antitoxin is of the "non-avid" variety. While calculations like these are not of general interest, you will see that the amount of antibody is both real and not yet large, for perhaps 6 ml. (or about 60 per cent) of the serum of the recipient guinea pig would be required in order to establish passively a state of fatal

anaphylaxis in a guinea pig, or the antibody contained in 18 to 30 ml. of the serum of a recipient rabbit. Further, knowledge of the exact amount of antibody allows us to make one important exclusion: the antibody that appears in the recipient could not have been masked *within* the transferred cells as an antigen-antibody complex. This follows from the fact that, even if one molecule of diphtheric toxoid (used as the antigenic stimulus) could bind 5 to 8 molecules of antitoxin, the amount of toxoid required would be so large as to reveal itself as an antigen transferred along with the cells, and there has been no such disclosure.

There has been one report linking human white cells with the appearance of antibody, for Walzer and Glazer have brought preliminary evidence of the transfer of human Prausnitz-Küstner reagins from man to man by means of cells. On occasion, the white cells harvested from the immunized animal will contain traces of antibody. Indeed, these small amounts have been the basis of the original studies on lymphocytes by Ehrlich and Harris and by White and Dougherty. Whether such traces may suffice to explain the transfer effects observed by Walzer and Glazer is not known. If these workers have demonstrated, rather, an actual local production of reagins, another substantial contribution will have been made to immunology.

How closely can we relate the cellular transfer of delayed type dermal hypersensitivity and the cellular transfer mechanism that leads to the appearance of antibody? I do not believe, myself, that a demonstration of circulating antibody can account for the manifestations of hypersensitivity of bacterial allergy, for example, which, as you know, is not transferrable by means of serum. Indeed, we have accumulating evidence against identification of the two phenomena; yet it seems evident that there exists a common principle. As Dr. Louis Dienes has repeatedly pointed out, we have no understanding of the incipient immunologic process. As a first guess, I suggest that both common antigenic stimuli (proteins, pollens) and stimuli which lead to delayed type hypersensitivity (bacteria, drugs) impress, although in different ways, the same type or types of cells that stand early in the chain of immunologic events. Whether the cells will give rise subsequently to either or both of the demonstrated end effects, and (if both) in what proportion, will be determined by the type or types of impress put upon these cells that I would view as "stem cells." The accruing evidence that every immunologic stimulus gives rise in some measure to both immediate type and delayed type components affords some justification for this view, in my opinion. At the same time, I do not want to convey the impression that lymphocytes are the cells that are chiefly involved in antibody production. It seems acceptable to view them as being associated in some way with the sequence of events that results in the production of antibody. Certainly, all recent research points to an intimate role of the plasma cell or other large cells of the lymphoid series with antibody production; due weight should not be denied to this other order of information merely through the circumstance that plasma cells are not under discussion today.

There remain to be considered the few facts available concerning the effect of hypersensitivity reactions on lymphocytes. The lymphocyte count, which is elevated in tubercular infection of the guinea pig and also in guinea pigs brought to sensitization by means of dead tubercle bacilli, is specifically depressed when the animal undergoes systemic reactions due to the injection of tuberculin in large amount. Dr. C. B. Favour has reported that in such a period, when tuberculous donors of cells have but few circulating lymphocytes, he has been unable to transfer tuberculin hypersensitivity by means of the white cells. In explanation of the disappearance of many lymphocytes in vivo, he would cite not sequestration of cells, but the phenomenon of specific "lympholysis," which he discovered in in vitro experiments. This phenomenon deserves more than today's passing mention, for ostensibly it is concerned with a special variety of antibody that occurs in the serum of tuberculous individuals, and the breaking up of lymphocytes (even those taken from normal individuals) shortly after this special antibody, tuberculin, and serum complement are added to them. This antibody, isolated in the form of gamma globulin concentrates, appears stable, whereas before isolation it was labile, and seemingly lacks any extraordinary characteristics. Lymphocytes removed from the tuberculous individual—mouse, guinea pig, or man—appear to carry a quota of this antibody, for they undergo proportionate lysis in vitro (up to 30 per cent) when only serum complement and tuberculin are provided. This basic observation, Favour's initial one, has been used by him with diagnostic advantage in certain instances of tuberculosis in man. The possibility that types of cells other than lymphocytes may also undergo disintegration in vitro in the presence of certain antibodies, antigen, and complement has been suggested by Byron Waksman in recent experiments. It remains an open question whether and to what extent the mechanism of the cell transfer of delayed type hypersensitivities will be found to be coordinated with aspects of the phenomenon of "lympholysis."

In this cursory review of the role of certain white cells, we have spoken of two phenomena that one encounters in studying the allergic manifestations of animals: (1) the role of these cells, which are probably lymphocytes, in transferring dermal hypersensitivity of both the delayed, contact type of reaction to drugs and of the delayed response to intradermal diagnostic reagents in bacterial allergies; and (2) the capacity of these cells to undergo specific lympholysis as described by Favour. The extension of these studies to man has given parallel results with respect to the transfer of bacterial allergy and to the occurrence of specific lympholysis. Initial experiments on the transfer of contact dermatitis in man by means of cells have shown that this technique does lead to the sensitization of the recipient in some instances, but interpretation of the new sensitization as a "passive" or "active" process, or both together, is still unsettled.

We have seen further that the same cells have a function also in the chain of events that leads to the appearance of circulating antibody. Upon transfer of cells from an antibody-producing animal, antibody is found that cannot be

detected within the cells themselves. Whether the antibody arises by means of metabolism of the transferred cells, or by transfer of some intermediate substance that is utilizable by the cells of the recipient animal, or is antibody held within the cells in some unknown, latent, or nascent form, has not been decided. The evidence is such as to urge the conclusion that the allergic and immunologic events are related, although distinct and separate in their appearance, and that they are related by means of a common cellular pathway.

DR. SAMTER (On Eosinophils).—The assignment to talk about eosinophils is at the same time simple and complex. Interest in eosinophils has been lively and persistent. In fact, looking at our audience, I can recognize at least eleven of you who have published at one time or another papers that involved eosinophils. Several months ago we made an estimate of the number of papers on eosinophils that have been published since 1879, when Ehrlich described their identification with acid stains. The total, plus or minus several hundred papers, is close to 10,000. In spite of this volume of work, our knowledge is deficient. I shall try to outline some specific areas of this deficiency.

The majority of investigators who have worked with eosinophils feel that the cells migrate into areas in which certain antigen-antibody reactions have taken place. One must emphasize the word *certain* because eosinophils are not participating in all antigen-antibody reactions. We must also qualify the use of the term *migrate into,* as a significant number of authors maintain that eosinophils do not migrate into, but are formed in and migrate out of, the tissues in which they are found. Each group is distinguished by a firm stand which is based essentially on morphologic evidence. An agreement appears unlikely until dynamic studies supplement and enhance the static foundations of our present convictions.

In the light of our recent advances in the understanding of the behavior of eosinophils, it is astonishing that many of the basic facts were known around the turn of the century. They have been forgotten during the interval. Ehrlich, for instance, mentioned in one of his early papers that circulating eosinophils disappear during an intercurrent infection, and recur during recovery. Ehrlich recognized clearly that their disappearance represented an absolute, not a relative, eosinopenia—the result of routine absolute eosinophil counts. Almost sixty years went by until Thorne recalled some of the previously described changes in the number of circulating eosinophils and rediscovered their usefulness as an index for adrenocortical activity. Since then, research on eosinophils has been sharply divided among investigators who are concerned with their increase and those who are involved in studies of the mechanics of their decrease in blood and tissues.

Our own group has concentrated essentially on the study of eosinophilia rather than eosinopenia. Eosinopenia, on the other hand, raises several intriguing questions. ACTH or, as it were, the cortisone-like hormones of the adrenal cortex, decreases the number of circulating eosinophils of a normal subject

within four hours to approximately 50 per cent of the pretreatment level. One wonders, of course, what happens to these eosinophils. Several possibilities have been mentioned:

1. They might be lysed in the circulating blood.
2. They might migrate into tissues—for instance, the spleen—and hibernate there.
3. They might migrate and be destroyed in the tissues.
4. They might stick to the wall of the vascular bed and wait patiently until the adrenocortical effect wears off and allows them to return into the circulation.

So far, the questions have not been answered, although many investigators (for instance, Essellier, Godlowski, Kark, Speirs, and others) have worked on various parts of the puzzle. The spleen has been ruled out; patients show the same decrease in circulating eosinophils after ACTH or cortisone before and after splenectomy. Kark and his associates incubated eosinophils in vitro with cortisone and found lysis of approximately one-half of the cells after four hours. The interpretation of their findings is complicated by the fact that cortisone appears to destroy approximately the same percentage of polymorphonuclear cells, a phenomenon that is in contrast to their behavior in vivo. Recently Essellier reported that he has been unable, under almost identical conditions, to confirm the lysis of eosinophils in vitro by cortisone; but it might well be that the word *almost* is a clue to the entire question. In our own fairly extensive studies on guinea pigs, we have shown that it takes an unexpectedly large amount of ACTH to depress the number of circulating eosinophils in normal animals of this species. Even larger amounts are required to depress the eosinophilia which develops in a guinea pig twenty-four hours after survival of anaphylactic shock. As a rule, however, lungs of such guinea pigs, which are removed at a time when the circulating eosinophil count is near zero or zero, show an abundance of eosinophils; this makes us reluctant to believe that eosinophils are destroyed in the tissues.

It is our suspicion that eosinophils adhere to the wall of the blood vessels during their eosinopenic phase, an opinion which is probably conditioned by years under the influence of my former teacher, Eliot R. Clark, who emphasized that "sticking" represents one of the basic controls in the distribution of moving cells.

Before leaving the field of eosinopenia, I would like to restate a fundamental problem for your consideration. If a patient has an absolute eosinophil count of 240 cells per cubic millimeter, the injection of 25 units of water-soluble ACTH produces, as a rule, a depression of approximately 50 per cent; in other words, the expected count ranges from 100 to 140 cells per cubic millimeter.

If a patient has an initial eosinophil count of about 500 cells per cubic millimeter, the same amount of ACTH will reduce it to an average of from 200 to 300 cells per cubic millimeter. Patients with high eosinophil counts appear

to conform to the same rule. A patient with 5,000 circulating eosinophils per cubic millimeter will respond more often than not, to the administration of 25 units of ACTH with a profound reduction; we have been able to demonstrate, in several patients who suffered from trichinosis, a disappearance of up to 5,000 eosinophils per cubic millimeter. If 25 units of ACTH can cause a disappearance of up to 5,000 eosinophils per cubic millimeter, it is difficult to understand why 25 units of ACTH given to a normal subject tend to reduce his circulating eosinophil count from 250 to perhaps 120, but not to zero. The strange half-life of the eosinophils is, I believe, one of the major areas which require clarification.

The bulk of the existing literature on eosinophilia essentially records its existence; there is a relative dearth of experimental work. Consequently, we do not know why eosinophils migrate into tissues toward the site of antigen-antibody reactions of the anaphylactic type. Transitory eosinophilia can be produced by other means (they appear in the gastrointestinal tract during digestion and in the circulation, in significant numbers, after the feeding of raw liver), but the allergic mechanism dominates the field. Several investigators have attempted to identify the chemotactic agent which is responsible for their occurrence in tissues after anaphylaxis. Last night Dan H. Campbell and I discussed studies which he had carried out years ago while he was still at the University of Chicago. At that time he tried to produce eosinophilia by injecting serum of guinea pigs which carried a large number of circulating eosinophils into normal recipient animals. In retrospect, it seemed unlikely to both of us that eosinophilia could be so transferred; if the still theoretical eosinophilic factor did circulate in the serum, there would be little incentive for eosinophils to leave the vascular bed and migrate into the tissues. On the basis of this reasoning, we have altered Campbell's original procedure. We sensitized, and shocked, guinea pigs with crystallized egg albumin. Within one to three minutes after anaphylactic death, we removed shocked lungs and transferred slices into the peritoneal cavity of nonsensitized recipient animals. Histologic examinations of shocked lungs demonstrate that they are free from eosinophils; this is to be expected, since death is too rapid to allow for cellular changes. Within twenty-four hours after transfer, however, the normal recipients of these slices show an increased number of eosinophils in the circulating blood. It appears that slices of lungs of shocked guinea pigs carry a substance which encourages the development of eosinophilia. While we are confident that this "eosinophilic factor" exists, we are in the dark about its nature. Attempts are on the way, however, to gain additional information and perhaps to isolate the active material.

In summary, we believe that the effect of antigen-antibody reactions of the anaphylactic type attract eosinophils to the site at which they occur. Whether this attraction is due to the antigen-antibody complex itself or to the changes which it precipitates (for example, activation of enzymes or changes in the chemistry of the tissue) remains to be seen. It should be stated, however, that the eosinophilic factor, more likely than not, represents a phase of the immunomechanisms which we as yet have not recognized; none of the partial functions which have been tested, e.g., liberation of histamine or heparin, attracts eosinophils.

A fair outline of the state of our knowledge of eosinophils might present some wistful blueprint for future work but reflects necessarily a considerable feeling of frustration. When Wharton Jones first described eosinophils as cells slightly larger than other white cells which carry double refractory granules in their cytoplasm, he added that their appearance was distinctive and their function unknown. Honesty requires us to state that there is very little, if anything, more than one can say at this point than Jones said 108 years ago.

Dr. B. Z. Rappaport: Dr. Samter speaks of the dispute as to the site of formation of eosinophils, whether in blood-forming organs or in tissue, and he favors the former. It seems to me that there is a third possibility that eosinophils may be produced both in the hemopoietic system and from undifferentiated cells in tissues. In studying allergic nasal mucosa, one sees suggestive evidence of the possibility of the tissue origin of eosinophils. While some of the cells are lobulated, suggesting an origin in blood-forming organs, many of the eosinophils have well-rounded nuclei. A fairly large proportion of the cells have multiple nuclei, each round and circumscribed, with no bridges to indicate lobulation. These findings seem to support the possibility that such eosinophils originate in the nasal tissue itself rather than in the blood-forming organs.

Another curious observation that one finds in allergic nasal mucosa is the large number of ruptured eosinophils. The eosinophil is hardier than the other granulocytes. Yet, one observes in allergic tissue many extracellular eosinophil granules, frequently in clusters, as if the cell had recently ruptured. It is interesting that no mention of this is made in the literature. Just recently I found in Cooke's textbook on *Allergy in Theory and Practice* an illustration of a histologic section of nasal mucosa containing a number of ruptured eosinophils. Interestingly, the state of the cell is not mentioned in the caption of the illustration or in the text. Possibly the reason for this is that we know nothing of the significance of the destroyed eosinophils.

Dr. Samter: Dr. Rappaport's remarks in support of a possible development of eosinophils in allergic tissues indicate the direction of the experimental problems which lie ahead of us. So far, neither the proponents of the "bone marrow only" theory nor the advocates of the "also in certain tissues" hypothesis of eosinogenesis have presented conclusive evidence for their respective beliefs, and one's opinion on the subject is rather speculative. I tend to side with Dr. Emil Schwarz, who, now in his late eighties, works at Michael Reese Hospital in Chicago. He feels strongly that after fetal life the tissues of man are unable to form eosinophils; in other words, eosinophils, wherever found, have migrated from the bone marrow to the periphery where they perform their mysterious function. I would feel better, however, if Dr. Dameshek would comment on the state of the controversy, and on his own position in it.

Dr. Dameshek: As this symposium draws toward its conclusion, let me say, first, that I have been greatly interested in listening to this exchange of ideas, as I have done a little in all the fields that the various speakers have discussed and I have benefited greatly from their discussion. Concerning the last question, I think you asked the wrong man, Dr. Samter, because I agree with you 100

per cent, and I cannot see any other way out. To me, it seems clear cut that the bone marrow produces various types of granulocytes, including the neutrophilic, the eosinophilic, and the basophilic granulocytes from the stem cell, the myeloblast, and on up through the various differentiating cells. The migration of eosinophils from the bone marrow through the blood into the tissues seems clear from various experiments, particularly those carried out with extracts of *Ascaris lumbricoides*. In these experiments, the development of pulmonary lesions simulating the Loeffler type of pulmonary syndrome is quite impressive. I have the impression that eosinophilia is evoked by certain more or less specific stimuli and that the eosinophils develop in the marrow and go out to the tissues where the stimulatory factor is present.

STUDIES IN PENICILLIN HYPERSENSITIVITY. II. THE SIGNIFICANCE OF PENICILLIN AS A CONTAMINANT

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SEVERE immediate reactions to the administration of penicillin, some ending in fatalities, are occurring with increasing frequency. Considerable attention has been directed toward methods of detecting the presence of marked sensitivity to penicillin in the patient so that its administration in such cases might be avoided. However, it has not been recognized up to this time that penicillin is commonly being administered, unintentionally, to many patients in amounts sufficient to produce severe reactions in the extremely sensitive patient.

In this communication, we are presenting a case report illustrating how a physician unknowingly induced a severe allergic reaction in a penicillin-sensitive patient by using a boiled syringe, which was contaminated with the antibiotic, for an injection of an unrelated substance. This occurred despite what appeared to be adequate washing and sterilization of the syringe.

The problem of contamination arises from the fact that it is difficult to wash syringes clean of penicillin and that some of the antigenic principles in penicillin remain active despite boiling for many hours. These findings provide a possible explanation for the mechanism of sensitization in those patients who deny a previous contact with penicillin, but develop severe reactions to the alleged "first" administration of this antibiotic. In addition, as is illustrated in this case, it raises some doubts as to the validity of the explanations for some allergic reactions which are reported and are attributed to injections of the most innocuous substances. It seems quite possible that in penicillin-sensitive patients some of the allergic reactions attributed to other drugs have in fact been due to penicillin administered unintentionally by the use of a syringe which had previously contained penicillin or had been boiled in a penicillin-contaminated sterilizer.

CASE HISTORY

Patient F., a 29-year-old Negro nurse, received an injection of penicillin in 1949 and two more during the next two years without any ill effects. In October, 1952, within fifteen minutes after swallowing a 200,000 unit tablet of penicillin, she developed itching of the palms, genitals, axillae, and soles of the feet. This was followed by itching of the throat and a generalized urticaria and angioedema. A subcutaneous injection of epinephrine relieved most of the symptoms within thirty minutes.

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A similar episode occurred on June 12, 1953, a few minutes after she was given an intramuscular injection which was purported to contain 25 mg. of an aqueous suspension of testosterone mixed with a small amount of 1 per cent procaine hydrochloride. This was being given for the correction of menstrual irregularity. In this reaction, the patient developed the symptoms previously noted and, in addition, tightness in the chest, audible wheezing, vomiting, and severe lower abdominal pains. There followed a brief period of syncope. After several injections of epinephrine, the urticaria and angioedema subsided, but the asthma persisted for about two hours. That evening the patient developed metrorrhagia.

On June 23, 1953, the patient's physician scratch-tested her with a 1 per cent solution of procaine hydrochloride and with a penicillin solution of unknown concentration. The reactions at both sites were reported to be positive. After an interval of about ten or fifteen minutes, intracutaneous tests were done with the same solutions. Almost immediately, marked reactions developed at both intracutaneous test sites. Within five minutes the patient complained of itching of the throat and suddenly lost consciousness. The skin was cold and the pulse imperceptible. After an injection of epinephrine, the patient rallied for a few minutes. She complained of severe tightness in the throat and chest. She again lost consciousness and remained in this state for almost two hours despite repeated injections of epinephrine. On regaining consciousness, the patient vomited and complained of severe asthma. This persisted with gradual diminution in intensity for twelve hours. During this period, metrorrhagia again developed.

Following this episode, this nurse experienced such symptoms as itching of the eyes, nose, and throat, swelling of the lips, and a tight chest whenever she handled penicillin or whenever it was used in her presence. This development compelled her to give up hospital employment and she resigned on Oct. 21, 1953. Since that day she has worked as a salesgirl and has remained symptom-free.

The patient's past history is negative for atopic illnesses. Her father had asthma and one brother has recurrent urticaria.

IMMUNOLOGIC STUDIES

The patient was referred to us for study on July 31, 1953. The cause of the allergic reaction which followed the intramuscular injection of testosterone and procaine was investigated. Because of the story of severe reactions following skin test with drugs, direct skin tests were avoided at first and early studies were made by the passive transfer technique.[1]

Passive Transfer Studies.—Sites on a normal subject were sensitized by intracutaneous injections of 0.05 ml. of the patient's serum (serum "F"). Four days later, separate sites were tested by the intracutaneous injection of about 0.01 ml. of a 1 per cent solution of procaine hydrochloride, and of an aqueous suspension of testosterone containing 25 mg. per milliliter. Negative reactions resulted. Another passively sensitized site on the same substitute was then tested with a solution of crystalline penicillin G containing 50,000 units per milliliter. Within ten minutes a marked reaction developed. A control test with the same penicillin solution on a nonsensitized site of the subject gave a negative reaction. These results were confirmed on several subjects.

In most subjects, all the untested, passively sensitized sites flared up within a few minutes after the initial tests with penicillin became positive. This phenomenon indicated that sufficient penicillin had entered the subject's circulation from the skin test sites to induce an allergic response at all passively sensitized areas.

Employing varying dilutions of penicillin, it was found that an intracutaneous test containing about 5 units of penicillin was sufficient to induce a flare reaction at a distant site sensitized with undiluted "F" serum. The spontaneous flare phenomenon may be interpreted as the equivalent of a "constitutional" reaction in the normal substitute.[2] It is an index of what might be expected following a skin test with even smaller amounts of penicillin in the patient.

The reagin titer of "F" serum was determined by the serum dilution technique of Coca and Grove.[3] The highest dilution of the serum that yielded a positive passive transfer reaction to a test solution of crystalline penicillin G containing 50,000 units per milliliter was 1:128. On sites sensitized with undiluted serum, the highest dilution of crystalline penicillin G which produced a minimal positive reaction (±) contained .0005 unit per milliliter. On similarly sensitized sites, a test with a penicillin solution containing .001 unit per milliliter gave a slight positive reaction (+). Further immunologic studies on this patient are reported in detail elsewhere.[4]

Results of Direct Skin Tests With Testosterone and Procaine.—Intracutaneous tests with the testosterone and procaine solutions in the concentrations previously described were then performed directly on the patient. They produced negative skin reactions and no constitutional symptoms.

Studies With Sterilizer Water.—The extreme degree of clinical sensitivity of the patient to penicillin, the presence of antibodies to penicillin in high titer in her serum, and the negative cutaneous and clinical reactions to tests with testosterone and procaine suggested the possibility that penicillin might have been present as a contaminant in the testosterone and procaine injection which she had received from her physician, and might have caused the constitutional reaction. Steps were taken to investigate this hypothesis.

Samples of water from the sterilizer of the patient's physician were obtained. The water in the sterilizer had not been changed since the patient had received her injection several months before, but, as is a common practice, had been repeatedly replenished and reboiled. When approximately 0.01 ml. of this water was injected into a cutaneous site which had been passively sensitized with serum "F," a slight positive reaction (+) was obtained. A slightly larger reaction resulted from testing with sterilizer water which had been drawn up into one of the physician's sterilized syringes. Normal unsensitized skin sites on the passive transfer subject gave completely negative reactions to tests with these materials.

TABLE I. RESULTS OF DIRECT INTRACUTANEOUS AND PASSIVE TRANSFER STUDIES IN PATIENT M. F.

TEST SUBSTANCE	CONCENTRATION	PASSIVE TRANSFER REACTIONS		DIRECT INTRACUTANEOUS TEST REACTIONS ON PATIENT
		ON SERUM "F" SITES	ON NONSENSITIZED SITES	
Crystalline penicillin G	500 units/ml.	++++*	0	Not done
Crystalline penicillin G	.0005 units/ml.	±	0	Not done
Aqueous suspension testosterone	25 mg./ml.	0	0	0
Procaine hydrochloride	1 per cent	0	0	0
Sterilizer water†		+	0	Not done

Passive transfer tests done with 0.01 ml. of solution four days after sensitization.
*Followed by spontaneous flare of distant untested sites.
†Obtained from sterilizer of patient's physician.

The results of these studies are summarized in Table I. They suggested that the severe reaction which the patient experienced following the injection of testosterone and procaine was not caused by either of these drugs, but in all likelihood by penicillin which was present as a contaminant in the physician's syringe. To substantiate this hypothesis it was necessary to study further the effect of heat on the antigen of penicillin.

Effect of Boiling on Penicillin Antigen.—Crystalline sodium penicillin G was dissolved in 1 liter of physiologic saline to make a final concentration of 50,000 units per milliliter. The solution was boiled for sixteen hours in a flask to which a reflux was attached to prevent evaporation of water and concentration of the solution. At various intervals,

specimens were drawn off and filtered through sterile Fiberglas filters to remove sediment. The samples were tested for antigenic potency by injecting about 0.01 ml. of each boiled specimen intracutaneously into a site passively sensitized with serum "F."

The results of tests with boiled specimens of penicillin are summarized in Table II. A test with unboiled solution gave an unusually pronounced passive transfer reaction (+++++) on a serum "F" site. The specimen boiled for ten minutes gave a marked reaction (++++) on a passively sensitized site. Specimens boiled for three and four hours gave moderate reactions (+++). The specimens boiled for six, eight, twelve, and sixteen hours gave slight to moderate reactions (++).

TABLE II. EFFECT OF BOILING ON THE ANTIGENIC ACTIVITY OF CRYSTALLINE PENICILLIN G SOLUTIONS

BOILING TIME	PASSIVE TRANSFER REACTIONS	
	ON SERUM "F" SITES	ON NONSENSITIZED SITES
0	+++++	0
10 minutes	++++	0
3 hours	+++	0
4 hours	+++	0
6 hours	++	0
8 hours	++	0
12 hours	++	0
16 hours	++	0

A vial containing 50,000 units of crystalline penicillin G per milliliter was autoclaved. A moderate passive transfer reaction (+++) was obtained by a test with this solution. Normal unsensitized skin sites on the subjects used in these passive transfer studies which were tested with the boiled and autoclaved penicillin specimens uniformly produced negative reactions.

Syringe Contamination Studies.—Since penicillin solutions retained some antigenic potency after sixteen hours of boiling, it became apparent that sterilizer water easily could become and remain contaminated with this antigen. Several methods of washing and sterilizing penicillin-soiled syringes were studied to see if such contamination could be prevented.

(a) All-glass syringes: Aqueous suspension procaine penicillin G in a concentration of 300,000 units per milliliter was selected for this study because of its wide clinical use in medical practice and because of its adherent properties. Two milliliters of this penicillin suspension were drawn up into a new, sterile, all-glass, 5 ml. syringe, with a needle attached, and then expelled. The syringe was then washed by removing the plunger and allowing running tap water to fill the barrel and needle. The plunger was then replaced and the surface of the needle was washed off with running water. This "washing" of needle and syringe was performed once, twice, or three times, depending on the experiment.

After washing, sterilization was performed in several ways. In one study, each all-glass syringe, with needle attached and plunger in place, was boiled for fifteen minutes in a separate, clean, water-filled beaker. In another study, the same procedure was repeated, but with the plunger outside the barrel. In a third study, each syringe, with parts separated, was sterilized by autoclaving. One syringe soiled with the penicillin suspension was left unwashed and was boiled with its plunger in place. After sterilization, 2 ml. of sterile physiologic saline was drawn into each syringe, which was then shaken well. Cutaneous sites sensitized with serum "F" and control nonsensitized sites on each subject were then tested with 0.01 ml. of each specimen of saline.

It was found that syringes which had been boiled for fifteen minutes with the plungers in place remained contaminated with penicillin even when they had previously been washed three times (Table III). The saline drawn through such syringes gave a slight positive (+) passive transfer reaction. From a similarly treated syringe that had been washed only

TABLE III. RESULTS OF PASSIVE TRANSFER TESTS WITH SALINE WASHINGS FROM PENICILLIN-CONTAMINATED SYRINGES

STERILIZING SYRINGE	TYPE OF SYRINGE	TYPE OF PENICILLIN USED	PASSIVE TRANSFER REACTIONS ON SERUM "F" SITES					
			SYRINGE UNWASHED	SYRINGE WASHED ONCE	SYRINGE WASHED TWICE	SYRINGE WASHED 3 TIMES	SYRINGE WASHED 6 TIMES	SYRINGE WASHED 10 TIMES
Boiled 15 minutes with plunger in barrel	All-glass 5 ml.	Aqueous procaine 300,000 u./ml.	++++	+++	++	+		
Boiled 15 minutes with barrel and plunger separated	All-glass 5 ml.	Aqueous procaine 300,000 u./ml.		0	0	0		
Autoclaved with plunger and barrel separated	All-glass 5 ml.	Aqueous procaine 300,000 u./ml.		++	0	0		
Boiled 15 minutes with plunger in barrel	"Allergy" 1 ml.	Aqueous procaine 300,000 u./ml.					+++	++
Boiled 15 minutes with plunger in barrel	"Allergy" 1 ml.*	Aqueous crystalline 50,000 u./c.c.					+	±

*Glass barrelled with asbestos-wound metal plunger.
Tests with above solutions on normal skin sites gave negative reactions.
Passive transfer tests with the diluent in aqueous procaine penicillin gave negative reactions.

twice before boiling, the saline produced a slight to moderate reaction (++). The saline from a boiled syringe washed once gave a moderate reaction (+++). From an unwashed boiled syringe, the saline gave a marked reaction (++++).

Contamination was more effectively eliminated when the barrel and plunger of the all-glass syringe were separated before boiling. Under these circumstances only one washing was sufficient to prevent contamination. Autoclaving of the separated syringe parts completely eliminated contamination only when the syringes had been washed two or more times. The saline from an autoclaved separated syringe which had been washed only once produced a slight to moderate passive transfer reaction (++).

(b) Asbestos-wound syringes: Additional studies were done with a type of 1 ml. syringe commonly employed in the practice of allergy, consisting of a glass barrel and an asbestos-wound metal plunger. It seemed likely that the presence of the asbestos packing and the fact that the syringe parts are not separated during sterilization would tend to increase the hazard of contamination with penicillin in this type of syringe.

One milliliter of aqueous suspension procaine penicillin G (300,000 units per milliliter) or a similar amount of aqueous crystalline penicillin G (50,000 units per milliliter) was drawn into a new asbestos-wound syringe. The penicillin was then expelled and the syringes washed six or ten times with tap water. Each syringe was then boiled in a separate, clean, water-filled beaker for fifteen minutes, with plunger in place in the barrel. After sterilization, 0.5 ml. of sterile physiologic saline was drawn into each syringe, which was then shaken well. The saline samples were then used for testing passively sensitized sites.

Saline from syringes which had been filled with procaine penicillin and then washed six times and boiled gave a moderate passive transfer reaction (+++). From such syringes washed ten times, the saline produced a slight to moderate reaction (++). With crystalline penicillin as the contaminant, the samples from syringes washed six times and boiled produced a slight passive transfer reaction (+) and, with ten washings, a negative to slight reaction (±). When tested on normal skin, all saline samples gave negative reactions. The diluent employed in the manufacture of the aqueous procaine penicillin was also tested on passive transfer sites and yielded negative reactions (Table III).

DISCUSSION

It is evident, from the preceding studies, that syringes are commonly contaminated with penicillin, and this hidden source of contact with the antibiotic may be responsible for allergic reactions in susceptible persons. Such a reaction occurred in the penicillin-sensitive patient reported here, following the use of a supposedly clean, boiled syringe for an injection of testosterone and procaine. Complete study by means of passive transfer and direct skin testing revealed negative reactions and no clinical responses to these two drugs. However, when the sterilizer water in which the syringe had been boiled was found to contain penicillin antigen in appreciable amounts, a logical explanation for the patient's severe allergic reaction to the injection of testosterone and procaine became apparent. It seemed likely that the contamination of the injectant with penicillin was responsible for the untoward reaction in this patient, who was extremely sensitive to the antibiotic.

It had been planned to inject the patient with another dose of testosterone and procaine, using a clean syringe, in order to prove that these preparations were harmless but the patient's permission could not be obtained for this test.

There are, in the medical literature, forty-three reported deaths and many more near-fatal anaphylactic reactions attributed to penicillin.[5-7] Measures have been recommended for the detection of severe sensitivity to penicillin and for

the protection of such patients from contact with penicillin.[8, 9] One must now add to such precautions the warning that such patients should not receive any type of injection unless it is certain that the syringe which is being used has not been contaminated with penicillin.

Allergic reactions caused by penicillin as a contaminant have not been reported to date. Some of the typical allergic reactions that have been attributed to drugs and medicaments which ordinarily do not act as allergens may well have been produced by penicillin administered in this manner. The severe reaction in the case reported here was originally attributed to procaine. The positive skin reaction to procaine obtained by the physician when he originally tested the patient undoubtedly was due to contamination of the testing syringe with penicillin. With uncontaminated syringes, we were unable to elicit positive reactions by direct tests with testosterone and procaine on the patient.

There is the possibility that patients who are receiving repeated injections of medication administered with syringes contaminated with penicillin may unknowingly become sensitized to the antibiotic in this manner. If the penicillin contaminant in a boiled syringe is sufficient to induce a severe constitutional reaction in the extremely sensitive patient, it is reasonable to assume that, in the susceptible atopic individual, the frequently repeated administration of small hidden amounts of penicillin, as contaminants of injections of other types of medication, may eventually result in penicillin sensitivity.

The minute amounts of penicillin present as a contaminant must be considered of antigenic significance when it is realized that 0.01 ml. of a penicillin solution containing 0.001 unit per millimeter, or a total of 0.00001 unit of penicillin, was found to be sufficient to produce a slight positive reaction (+) on an "F" serum-sensitived site. It is noteworthy that this amount represents only one thirty-billionth ($\frac{1}{30,000,000,000}$) of the usual therapeutic dose employed in practice (300,000 units). The amount of antigen found in some contaminated syringes is many times greater than this.

Several authors, in discussing the delayed skin reaction as a manifestation of penicillin sensitivity, have stated that 4 to 5 per cent of the population demonstrated "spontaneous" sensitivity to this drug.[10, 11] This opinion must be re-evaluated in view of the frequency with which patients are exposed unknowingly to penicillin, by injection, inhalation, contact, or ingestion. It is possible for susceptible persons to become sensitized by the inhalation of traces of penicillin.[4] Penicillin is present in the milk of cows receiving this antibiotic.[12] Such hidden contacts and routes of sensitization undoubtedly explain some of those puzzling cases of penicillin sensitivity which are said to occur with the "initial" contact with the drug.

The contamination of syringes with penicillin is a problem primarily because boiling does not destroy its antigenic property. When a solution of penicillin G was boiled for sixteen hours, its antigenic activity was still pronounced and could be readily demonstrated by passive transfer tests.

The effect of boiling on the antibiotic activity of penicillin also was studied and will be reported in detail in another communication. While boiling produced a rapid fall in antibiotic potency, complete inactivation was not effected and traces of antibiotic activity could be detected after sixteen hours of boiling.

One of the most important practical considerations in the problem of penicillin contamination concerns the need for cleansing syringes adequately. It has been demonstrated that an all-glass syringe, which had been used for penicillin, retained traces of the drug even when it had been washed three times with tap water and then boiled with the plunger left in the barrel, as is a common practice. An asbestos-wound syringe remained contaminated despite ten washings prior to sterilization. Apparently, boiling a syringe with the plunger in position does not allow for adequate circulation of boiling water inside the barrel and prevents the removal of contaminating particles of penicillin. Asbestos wrapping retains large amounts of penicillin and increases the degree of contamination. Furthermore, if syringes contaminated with penicillin are placed in the sterilizer, the water in the sterilizer becomes contaminated and remains so even after many hours of boiling and reboiling.

To reduce the likelihood of contaminating syringes and sterilizers with penicillin, it is imperative that all-glass syringes and needles be washed at least three times with running water after using. The syringe parts should then be separated before they are placed in the sterilizer. Sterilizers should be cleaned often and the water in them should be changed frequently and completely. It would be advisable to reserve separate syringes and a separate sterilizer for use with penicillin. Another solution to the problem might be the use of disposable cartridges in connection with penicillin administration.

Once an asbestos-wound syringe has been used for penicillin it must be kept separate, since it is almost impossible to eliminate the penicillin with the usual methods of washing and sterilizing until new packing is inserted. Contamination of other syringes and antigen solutions is likely to result unless this precaution is taken.

SUMMARY AND CONCLUSIONS

1. A case is reported in which penicillin, as a syringe contaminant, induced a severe immediate allergic reaction in a highly sensitive patient.

2. Contamination of syringes and sterilizer water with penicillin presents a serious problem, as it is difficult to wash the syringes clear of penicillin, and prolonged boiling does not completely inactivate the antigenic principle of the drug.

3. It is hazardous to administer any type of injection to patients who are known to be markedly sensitive to penicillin unless it is certain that the syringes to be employed are not contaminated with penicillin.

4. Allergic reactions in penicillin-sensitive patients, which are commonly attributed to nonantigenic drugs, may in fact be caused by penicillin as a syringe contaminant.

5. This case report suggests the possibility that sensitization to penicillin may result from repeated injections of unrelated substances with syringes contaminated with penicillin.

6. Recommendations for the prevention of contamination of syringes with penicillin are presented.

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THE ACTIVITY OF HUMAN REAGINS EXPOSED TO THE DISINFECTANT, BETA-PROPIOLACTONE*

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WHILE examining human serum for the presence of reagins by the Prausnitz-Küstner technique, one may transmit homologous serum jaundice to the recipient. Although the transmission of hepatitis virus type B is usually associated with the intramuscular or intravenous injection of human blood or some of its products, there is little reason to doubt that the intradermal injection of such materials, even in minute amounts, may result in hepatitis in the recipient. Loveless[1] observed a subject who developed viral hepatitis subsequent to repeated passive transfer skin testing with human sera. Also, the tattooing needle has been incriminated in the occasional transmission of viral hepatitis.[2, 3]

Ultraviolet irradiation of infected plasma under suitable conditions apparently inactivates the virus.[4, 5] Exposure of serum or plasma to such irradiation does not appreciably alter the electrophoretic patterns or solubility of the protein components,[5, 6] nor does it inactivate serum reagins.[1] However, recent studies indicate that ultraviolet irradiation is not always a reliable means of rendering plasma noninfectious with respect to hepatitis.[7-10]

Storage of plasma for prolonged periods at room temperature may prove to be a thoroughly effective means of inactivating serum hepatitis virus[11-13] but, due to the known heat lability of many reagin specimens and to the desire to study reagins within a reasonably short period after obtaining the blood sample, it was considered unprofitable to explore this method of hepatitis prevention.

Hartman and his associates[14] reported that the compound, beta-propiolactone† (betaprone), when added to plasma samples in a concentration of 0.15 per cent, inactivated seven unrelated viruses present therein. Hepatitis viruses were not included among those tested. At a concentration of 0.2 per cent the compound also killed six diverse species of bacteria in serum samples. Murray,[15] studying the action of beta-propiolactone on hepatitis B virus in plasma, has indicated that the compound appears to reduce the infectivity of a given dose of the virus for human volunteers. Betaprone hydrolyzes rapidly in serum and its degradation products are relatively nontoxic for animals.[14, 16, 17]

Hartman's[14] report on the favorable disinfectant action of betaprone prompted a study of the possible inactivating effect of this compound upon serum reagins with the aim of using the compound to reduce the risk of viral hepatitis attending Prausnitz-Küstner skin testing. Evidence is presented here which indicates that betaprone does not produce appreciable loss of reagins in treated serum.

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†Manufactured by the B. F. Goodrich Chemical Company, Cleveland 15, Ohio.

METHODS

Beta-propiolactone,* a colorless liquid, was stored in a refrigerator. Just prior to use it was mixed with sterile saline to yield a solution of known and convenient concentration. This stock solution was then added aseptically to a quantity of serum so that the volume increment was small and the final concentration of betaprone was 0.2 per cent by volume. Precautions were taken to insure thorough mixing of the betaprone and the serum, even with the minute aspiration droplets of serum produced by expelling the serum from the delivery pipette on the upper wall of the tube. No pH adjustment of the mixture was made, but with some serum samples the pH was measured with a glass electrode before and after treatment. The betaprone-treated serum was then incubated at 37° C. for two to three hours in a water bath. An aliquot of the serum so treated was cultured in Bacto brain-heart infusion medium (Difco Laboratories, Inc.) containing 0.1 per cent agar. The cultures were examined for growth after incubation at 37° C. and at room temperature for three days. Serum dilutions were made after treating the serum with the disinfectant.

For comparative purposes in one case, serum was mixed with cold liquid ethylene oxide, a potent germicidal agent, at a final concentration of 6 to 7 per cent. The mixture was incubated at 37° C. for twenty-four hours, treated with sterile sodium thiosulfate solution to reduce the residual ethylene oxide, and stored until tested.

The sera under test were derived from hay fever patients. Nonatopic persons served as recipients in the Prausnitz-Küstner tests. Approximately twenty-four hours intervened between serum injection and the subsequent antigen injection into the same site. In all cases an excess of antigen was injected in order to bring out the maximal reaction. The wheal and the erythema were measured separately along two perpendicular diameters and these were then averaged to give a single dimension for each.

RESULTS

Passive transfer skin reactions obtained with treated sera were compared with reactions produced by controls in which saline was substituted for the disinfectant in the serum. Table I presents the data derived from a study of three human sera. There appears to be little or no inactivation of the reagins present in the betaprone-treated sera, judging from the size of the reactions developed by corresponding test and control sites. In Experiment IV, the reactions with betaprone serum are somewhat smaller than with the saline serum. This difference, of course, could be due to some inactivation of the reagins or to technical and chance error; experience suggests the latter. Experiment V indicates that ethylene oxide, at the concentration employed, inactivated most, if not all, of the reagin in the serum.

Since these initial observations were made, betaprone has been used routinely with all sera in human passive transfer tests as a safety precaution. Reagin titers of these sera from hay fever patients have varied from zero in a few instances

*One sample of this compound was generously supplied to us by Dr. J. Spizizen of Sharp and Dohme, Inc., West Point, Pennsylvania. Dr. Spizizen originally drew our attention to the activity of beta-propiolactone. A second sample was very kindly furnished by the B. F. Goodrich Chemical Company.

TABLE I. COMPARISON OF PRAUSNITZ-KÜSTNER TEST REACTIONS GIVEN BY DISINFECTANT- AND SALINE-TREATED SERA

EXPERIMENT NO.	TEST SERUM	SERUM DILUTION	ANTIGEN	MEAN DIAMETERS OF WHEAL AND ERYTHEMA (MM.) SERUM + DISINFECTANT: DISINFECTANT (PER CENT)	WHEAL	ERYTHEMA	SERUM + SALINE: WHEAL	ERYTHEMA
I	A	1:1	Timothy	BP-0.1	14	37	13	36
			Timothy	BP-0.2	14	37		
II	B	1:1	Timothy	BP-0.2	12	39	11	38
		1:10	Timothy	BP-0.2	10	36	10	32
		1:50	Timothy	BP-0.2	0	0	0	0
III	C	1:1	Ragweed	BP-0.2	12	40	12	39
		1:10	Ragweed	BP-0.2	10	31	10	30
		1:50	Ragweed	BP-0.2	8	20	—	—
IV	C	1:1	Ragweed	BP-0.2	10	29	15	38
		1:5	Ragweed	BP-0.2	8	18	10	27
V	C	1:1	Ragweed	EO-6	0	22	14	45

BP, beta-propiolactone; EO, ethylene oxide. 1:1 dilution signifies no dilution (except for that produced by the additive, amounting to a serum dilution of 9:10). Each reaction figure represents the mean of two perpendicularly measured diameters.

to greater than 1:500. In most of these cases where the serum had a zero titer of reagin to a particular antigen it also had a relatively high titer of reagins to another antigen under test. Antigens employed were those indicated by the person's clinical history and skin test results.

Ethylene oxide at 6 per cent concentration caused a precipitate to form in the serum. This has not been observed so far with betaprone. However, 0.2 per cent betaprone did cause a precipitate to form in clear extracts prepared from human aorta.

Recently, a serum specimen was encountered which had its reagin activity markedly reduced by incubating the serum at 37° C. for two hours with or without added betaprone. A sample of serum obtained previously from the same atopic patient did not show this marked temperature lability of its reagins. Further studies would be necessary to determine whether or not treatment of the serum with betaprone at 6 to 10° C. for twelve to twenty-four hours (conditions which Hartman and his co-workers[14] found satisfactory for destroying their viruses) could be substituted in this case.

TABLE II. pH VALUES OF SERUM SAMPLES BEFORE AND AFTER TREATMENT WITH BETA-PROPIOLACTONE

SERUM	BEFORE TREATMENT	AFTER BETAPRONE TREATMENT: INITIALLY	AFTER BETAPRONE TREATMENT: AFTER TWO HOURS' INCUBATION	SERUM	BEFORE TREATMENT	AFTER BETAPRONE TREATMENT: INITIALLY	AFTER BETAPRONE TREATMENT: AFTER TWO HOURS' INCUBATION
A	8.45	7.05	6.25	H	7.9	6.9	6.35
B	8.1	6.85	6.0	I	8.15	7.05	5.55
C	8.3	6.8	6.3	J	8.05	6.85	5.75
D	7.3	6.7	6.55	K	8.3	6.75	5.80
E	8.3	6.85	6.35	L	8.35	6.75	5.60
F	7.85	6.85	6.3	M	8.35	6.85	5.75
G	8.05	6.9	6.2	Average	8.11	6.86	6.06

Hartman and his associates[14] apparently adjusted their serum and plasma samples to pH 6.5 before adding betaprone because of the more rapid hydrolysis of betaprone on the alkaline side of neutrality. The effect upon pH of adding betaprone to a final concentration of 0.2 per cent in serum was studied and the results were recorded in Table II. Measurements of the pH were made before and immediately after adding 2.0 per cent betaprone solution (dissolved in physiologic saline) and again after a two-hour incubation period at 37° C. in cotton-plugged tubes. Prior to testing, sera had been stored at –20° C. in rubber-stoppered vials for varying periods. Before treatment, the sera had a pH range of 7.3 to 8.4. Immediately after betaprone addition, the extreme values ranged from 6.7 to 7.05, with an average value of 6.86. Following the two-hour incubation period, the pH was found to be still lower, ranging from 5.55 to 6.55, with a mean value of 6.06.

An early sample of betaprone was kept at room temperature. After some months of intermittent use, it lost its ability to dissolve completely in saline. Gresham and his associates[18] have studied some of the chemical properties of this compound and found that it polymerizes, slowly under some conditions and readily under others, to form water-insoluble complexes. Another sample stored in the dark at 4 to 10° C. for over one and one-half years, so far, has retained complete solubility in saline solution in the region of 2.0 per cent concentration.

Betaprone was reported to be not only a viricide, but also a bactericide.[14] Although the sera listed in Table I were Seitz filtered before use, following this early experience the routine processing of sera for Prausnitz-Küstner testing has not included such filtration to insure sterility. Reliance has been placed on the betaprone treatment with subsequent culturing of the treated serum. So far all sera studied have been culturally negative. Most of these sera have been collected under reasonably aseptic conditions, but some were processed under conditions adverse for maintaining sterility and yet, following betaprone treatment, they have shown no evidence of living bacteria.

DISCUSSION

On the basis of the limited experience reported, beta-propiolactone does not appear to appreciably inactivate human serum reagins. Also, since its toxicity is low,[14, 16, 17] there seems to be no obvious contraindication for its employment in sera under scrutiny for reagin activity. Whether or not betaprone will fulfill its primary duty of inactivating hepatitis viruses in serum has not been completely established as yet. Also, it is not possible to state definitely at this time that the concentration of this agent used here, 0.2 per cent, is optimal for hepatitis B virus inactivation. Judging from Hartman's report[14] dealing with viruses other than those causing hepatitis, the minimal concentration of beta-propiolactone causing inactivation of a virus in serum depends upon the virus concentration and probably the pH. Thus, the recommended concentration of beta-propiolactone might be satisfactory for most hepatitis virus-infected serum samples but not for occasional ones possessing very high concentrations of virus.

Measurements of pH made on serum samples treated with betaprone to a concentration of 0.2 per cent indicate that betaprone will markedly reduce the

pH of serum, usually to values below pH 7.0. This reduction of pH probably is due to hydrolytic products of beta-propiolactone occurring as impurities or to rapid hydrolysis to some degree in the fresh 2.0 per cent solution in saline. Upon incubating the serum-betaprone mixture, there is a further drop in pH, probably due to continued hydrolysis of the compound. Within one minute of preparation the pH of a 2.0 per cent stock solution of betaprone is 3.3 in either water or 0.85 per cent sodium chloride solution. If this effect is due to hydrolytic impurities in the reagent before adding water, it may vary in degree with the age of the reagent. This hydrolytic process introduces a degree of uncertainty into any statement of concentration of beta-propiolactone in serum. In making up the stock solution of betaprone and also in adding the stock solution to the serum, an attempt was made to err slightly on the side of adding excess betaprone rather than to have the calculated concentration less than 0.2 per cent in the serum. These experimental conditions may or may not match those which ultimately are found best suited for inactivating hepatitis viruses in serum or plasma.

As a secondary consideration beta-propiolactone shows promise as a substitute for Seitz filtration of serum to render it free of living bacteria prior to skin testing. It would seem, however, that its use here would be limited to sera contaminated with nonsporulating bacteria, since spore formers are notorious for their resistance to many of the chemical disinfectant agents.

SUMMARY

The viricidal and bactericidal agent, beta-propiolactone, when admixed with serum in a concentration of 0.2 per cent, does not destroy reagins appreciably. The treatment of serum for Prausnitz-Küstner testing with this agent is recommended as a possible safety measure directed especially toward transmission of viral hepatitis. At this time, although there is some evidence from other sources that beta-propiolactone does inactivate hepatitis B virus in serum, the viricidal action of this compound on this particular virus has not been completely elucidated.

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INTERPRETATION OF THE INEFFICACY OF ANTIHISTAMINICS UPON THE SECRETION OF GASTRIC JUICES

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THE role of histamine in the secretion of gastric juice is not yet explained and there is no evidence for its identification with the gastrin.[1] It is very probable that histamine is of some physiologic importance, at least, in relation to the secretion of hydrochloric acid in the stomach. Therefore, histamine might have some significance in the genesis of peptic ulcer. It was shown by Halpern and Martin[2] that if one protects guinea pigs from the lethal action of histamine with antihistaminics it is possible to induce in these animals gastric ulcers which may even perforate. It is a fact that diamine oxidase may destroy the secretory principle of the extract of the stomach.[3] It has been stated often that the ulcer is of allergic origin, but this view is not accepted by all scientists. Some authors state that ulcer may be compared with the Arthus phenomenon.[4] It is not possible to produce evidence that the content of histamine in the blood of patients with gastric ulcer is increased. This may indicate that the quantity is too small for exact determination. There is some indirect evidence that blood histamine is increased in patients with gastric ulcer, for these patients exhibit an increased capillary permeability.[5] It is important to point out that the allergic reaction is generally accompanied by increased tone of the parasympathetics;[6] that alone, without the action of histamine, may cause increased secretion of gastric juice.

Soon after the introduction of antihistaminics into therapy, studies were made to determine whether or not drugs of this group prevent the increased secretion of gastric juice associated with gastric or duodenal ulcers. We can state, generally, that a great majority of the authors who have investigated this problem have come to a negative result.[7] There are some positive reports, however, but probably they can be ascribed to the local anesthetic effect of the antihistaminics and not to the proper antihistaminic action. It is very significant in this respect that Pyribenzamine prevents this effect when given orally but not when given by parenteral application.[8] The local anesthetics alone do not prevent the action of secretagogues.[9] Last, it should be remembered that many of the antihistaminics in higher dosage are also sympathicolytic, which likewise may prevent the secretion of gastric juice.

The question is raised as to why the antihistaminics do not prevent the secretion of hydrochloric acid caused by the injection of histamine. We have stated already that under physiologic conditions histamine probably stimulates the secretion of hydrochloric acid. We may attempt to explain this on the

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basis of our previous work and on the basis of new findings obtained from histochemical determinations of diamine oxidase. As far back as 1946, we set forth the thesis that the antihistaminics act by means of inhibiting diamine oxidase. According to our investigations, diamine oxidase is a receptor for histamine. Many experiments forward this assumption. It is a matter of fact that in the absence of diamine oxidase there is neither allergic reaction nor sensitivity to histamine. It is known that the myocardium does not contain diamine oxidase[11] and the heart cannot be sensitized.[12] The rat is generally poor in diamine oxidase and it is almost insensible to histamine, notwithstanding the fact that it contains histamine in normal quantities.[14] We demonstrated that the uterus of the rat does not contain diamine oxidase and it is known that this organ of the rat reacts to addition of histamine with relaxation, and not with contraction as in the case of all other smooth muscle organs. We could show, too, that the gravid uterus of the rat has a high content of diamine oxidase and that such a uterus reacts to histamine with contraction, which can be prevented by antihistaminics.[15] We could demonstrate that the same is true for identical reasons in the uteri of rats deficient in thiamine. It is known that the lungs of the rat contain only small quantities of diamine oxidase[16] and we were able to show that in most instances they react to histamine with bronchoconstriction, which also can be prevented by antihistaminics.[17]

On the basis of the characteristics of diamine oxidase extensively described by Zeller,[18] we should expect that the antihistaminics, especially those belonging to diamino series, would inhibit this enzyme. The diamines with a short carbon chain, such as the antihistaminics, Antergan, Antistin, and Pyribenzamine, are better bound to diamine oxidase, but diamine oxidase cannot destroy them, whereas the diamines with longer carbon chain, such as cadaverine or histamine itself, are loosely bound to diamine oxidase, but it destroys them more quickly. This may be seen from the fact that, if an antihistaminic is added to an isolated organ, it is washed away with difficulty, whereas histamine is washed away easily. It may be mentioned, however, that monoamines also inhibit diamine oxidase. This is important to know, because some of the antihistaminics, such as Benadryl, are derivatives of monoamines. This inhibition of diamine oxidase by antihistaminics was demonstrated indirectly in the quoted experiments of myself and of others. We have pointed out as a most important feature that the antihistaminics enhance the content of histamine in the blood of healthy and allergic persons.[19] If diamine oxidase is not inhibited, it may decrease this "intrinsic" histamine which is formed in allergic reaction. It is also in full accordance with the finding that an organ where the allergic reaction has taken place contains less histamine after than before the shock occurred.[20]

Our last experiments with Zeller in his department in Chicago showed that diamine oxidase, in fact, can be inhibited by antihistaminics, even though other authors who previously attempted this could not prove it.[21] These authors used a watery tissue extract and we made use of a sediment obtained by centrifuging a watery suspension. Strehber[22] had previously obtained

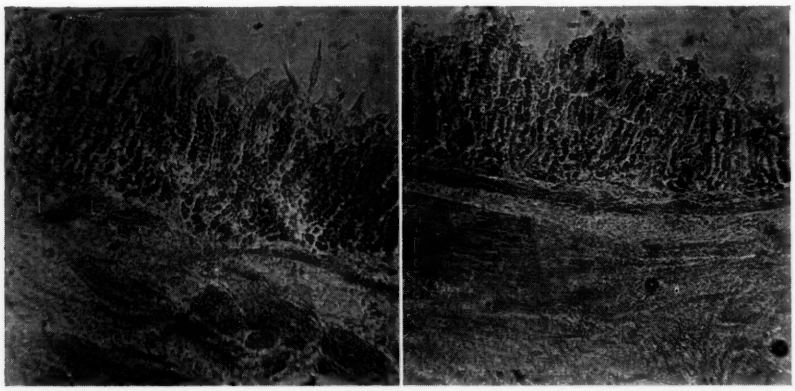
similar results by determining the activity of diamine oxidase in kidneys of rabbits with the Masugi type of nephritis after giving antihistaminics in vivo. These observations of Zeller and our own observations will be published elsewhere.

Best and McHenry[11] did not find diamine oxidase in the heart or skin; however, it was found later[23] in the skin and in the stomach. Therefore, according to our thesis, it is obvious why the myocardium cannot be sensitized and also why the antihistaminics do not prevent the secretion of gastric juice, for if there is no diamine oxidase the antihistaminics do not have anything to act upon.

The problem is complicated by the fact that the stomachs of animals and human beings react to histamine with contraction.[24] The stomach may display also obvious allergic changes which can be inhibited by antihistaminics.[25] The stomach, therefore, must also contain diamine oxidase. We succeeded in explaining this contradiction to our thesis by histochemical determination of diamine oxidase according to the method of Valetta and Cohen.[26]

METHOD

We made use of rabbit stomach. Nearly thirty healthy rabbits of both sexes, weighing 1 to 2 kg.,were killed by a blow on the neck and immediately thereafter their stomachs were removed and washed out. The parts nearer to



A. *B.*

Fig. 1.—*A*, Section of stomach wall; dark spots in muscularis record diamine oxidase. *B*, Section of the same stomach, stained without histamine; muscularis without dark spots.

the cardia were cut out, frozen with carbon dioxide without fixation, and cut into sections about 40 micra thick, the knife also being cooled. The sections were put in a solution of 2 per cent sodium bisulfite for twenty-four hours with an 0.5 per cent solution of histamine prepared with a buffer of pH 7.2 and controls under the same conditions without histamine. After washing with distilled water, they were put for one hour in Feulgen's reagent and then rinsed with 2 per cent solution of sodium bisulfite. The tissues which contained diamine oxidase showed islands of dark purple color, whereas other parts were of pink cyclamen color (Fig. 1).

As it can be seen from Fig. 1, the mucosa of the stomach does not contain diamine oxidase, whereas the muscular coat and the muscularis mucosae show islands which have the staining reaction of diamine oxidase. Therewith, we can explain why the antihistaminics do not prevent the secretion of gastric juice and why histamine nevertheless elicits a contraction of the wall of the stomach. These histochemical observations confirm our finding that both histamine and antihistaminics are bound to the same receptor, namely, diamine oxidase. We might mention here that we were able to confirm the finding of Best and McHenry[11] that the myocardium does not contain diamine oxidase but the epicardium and endocardium do contain it. Where there is no diamine oxidase, there is no activity of histamine. An antihistaminic displaces histamine from its receptor. This is easy to understand when we know the qualities of diamine oxidase as interpreted by Zeller. The question may be raised as to why Best and McHenry did not find diamine oxidase in the stomach. The techniques employed were probably responsible for that. They could not demonstrate diamine oxidase in the skin, but later it was demonstrated there. It may be remembered that during the mincing of the tissue diamine oxidase can come into contact with inhibiting substances such as vitamin B_1. That is why Zeller recommended the dialysis of this enzyme during the preparations for the experiment. In the histochemical determination, which we used, this factor does not come into play.

If we analyze our results and if we compare them with facts stated in the introduction of our paper, we again come to the conclusion that diamine oxidase is a receptor for histamine. The phenomenon of an enzyme which splits some definite substrate may be simultaneously its receptor is not a unique case in nature. For a long time this has been established for adenosine triphosphate and adenosintriphosphatase.[27] For more than ten years we have interpreted in this way the action of acetylcholine and of cholinesterase. The cholinesterase also, according to our opinion, is a receptor for acetylcholine,[28] as was clearly formulated recently by Zupancić.[29]

Thus, our experiments give support to the view that the action of histamine on the secretion of gastric juice differs from other histaminergic actions in nature.

SUMMARY

By means of a histochemical method, it was shown that gastric mucosa does not contain diamine oxidase, in distinction from muscular parts of the stomach. We think that diamine oxidase acts as a receptor for histamine and histamine cannot act without diamine oxidase. The antihistaminics inhibit diamine oxidase also by displacing histamine. In this way, we can explain why the antihistaminics cannot prevent increased secretion of gastric juice, that is, why they cannot cure peptic ulcer.

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Editorial

EVALUATION OF NEW DRUGS

THE accelerating pace of modern science is nowhere more manifest than in the development of new therapeutic agents. The pharmaceutical manufacturers are overlooking no possible source, from the investigation of the neglected primitive drugs, such as Rauwolfia, to the fresh field of mycology, which has produced so many antibiotics. Even more striking is the creation of new agents by chemical synthesis. Determination of the chemical structure of natural drugs and hormones has been quickly followed by the production and study of related compounds which, in some cases, have proved more useful than their prototypes. In addition, patient chemical and pharmacologic research has produced whole groups of new drugs, such as the sulfonamides and antihistamines, for which no natural models existed.

This flood of new agents has revolutionized the treatment of many important diseases. While the practicing physician rejoices in this succession of valuable new tools, he may well be confused by the rapidity with which they are showered on him.

Among the many new drugs brought to his attention by a profusion of advertising circulars and detail men, some represent notable advances in therapy. Others probably will be discarded in a few months, but recognizing which is which is not easy at the start. In other cases, as with the antihistamines, such a number of good but similar compounds are offered that individual judgment of relative merit is practically impossible.

While the practitioner may derive some comfort from the activities of the Food and Drug Administration in screening out unduly dangerous compounds, and the Council on Therapy of the American Medical Association in eventually accepting the notable new drugs, many of the compounds to which his attention is attracted, especially in such prolific fields as the antihistamines, have not yet been considered by the Council. Naturally, he turns for guidance to the medical journals. The JOURNAL OF ALLERGY, in common with other medical journals, recognizes this as an important type of information to convey to its readers. However, the number of articles dealing with new drugs which are submitted far exceeds the space available. Selection for publication is made on the basis of both merit of the study and presumed interest to the reader. Thus, preliminary studies on metacortandracin (Prednisone) may be chosen in preference to more thorough studies of less interesting compounds.

A considerable proportion of papers submitted deal with new drugs added to such already crowded fields as the antihistamines and the epinephrine group. While some of these compounds eventually may prove to be valuable additions, judgment is difficult. It is the opinion of the editor that worth-while evaluation of the relative merits of new drugs in such fields can be better accomplished by groups of experienced investigators, such as the Committee on New Drugs of the American Academy of Allergy, than by individual physicians or small clinics. This committee is guided by able pharmacologic consultants and has established a careful program for investigation of drugs submitted to it.

Without attempting to discourage individual research or deprive investigators of due priority in notable advances, it is suggested that physicians interested in routine studies of new drugs for allergic disease work in conjunction with the committee and that manufacturers producing compounds for use in this field submit them to the committee.

American Academy of Allergy

PRESIDENT'S ADDRESS

JOHN M. SHELDON, M.D.
ANN ARBOR, MICH.

UNDERGRADUATE MEDICAL EDUCATION IN ALLERGY

A FEW years ago this period was set aside for your president to attempt to justify his presidential existence. It is my sincere hope that each one of you some day will be on the same spot, and I am sure that everyone who has preceded me can well attest that it is a unique experience. To have been honored by election to the presidency of a society which has been an important part of my professional life has produced in me an even more intense feeling of closeness to it and appreciation of it. If in the past I had thought as seriously about our organization as I have been forced to do this year, I am sure that I would have derived the same benefits without the high office. I only hope that all of you will give serious thought to the future of our organization, for it is only through such a medium that we can hope for the stimulus, the principle, and even the life of allergy.

I propose today to discuss a subject that I think is of the utmost importance for the future of allergy—that is, *medical education*—and then it will be my great pleasure and honor to present a gavel as a token of appreciation to the past presidents of our two founding organizations and to the past presidents of the American Academy of Allergy.

May I present first what I believe are some of the legitimate reasons for considering the need for education in allergic disease. I think that all of us will agree that instruction of the undergraduate medical student in allergic problems has lagged behind the organized presentation of other subjects in the field of medicine. The increasing awareness of allergic disease as a source of chronic, debilitating illness has compelled a number of medical schools to examine their present methods of teaching the subject of allergy, and I am glad to report to you today that all but five medical schools in the United States now have separate sections of allergy for the purpose of instructing undergraduate medical students. In many instances, however, there is insufficient time allotted to the subject and very spotty integration with other sections and departments of the medical schools. Yet, there is little question about the

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need for adequate familiarity with clinical manifestations of hypersensitivity. The severe reactions to penicillin and other antibiotics, as well as the serum sickness response to the administration of horse serum in a sensitized patient and the multiplicity of drug reactions, are definite allergic conditions which need to be anticipated and treated properly when they occur. Likewise, the vast numbers of patients with nasal, pulmonary, and skin allergy require that physicians everywhere be well versed in the diagnosis and treatment of such conditions. In the past, there has been a tendency to relegate to the basic science instructors the teaching of manifestations of hypersensitivity and immunity, with no emphasis on correlation between the experimental phenomenon in animals and its counterpart in man. In the years of clinical instruction, a student may encounter on the wards or in the clinics a certain number of allergic patients who present themselves for treatment. To depend solely upon this instruction almost always results in a student's failure to obtain adequate formal instruction in those aspects of allergic disease which are not represented by those patients encountered. Likewise, to permit hit-or-miss assignment in the class will result in some students' seeing very few cases of allergy, while others may, by chance, obtain adequate experience with these conditions. There is a tendency, when the instruction in allergic diseases is left up to several departments, for little time to be devoted to the subject, resulting in a disproportionate lack of emphasis in relationship to the clinical importance of allergic disease. When four or five departments share the responsibility for instruction in allergy, the adequacy of such instruction all too often depends entirely on the extent of the individual instructor's interest in the subject. As one result of this kind of teaching, it is rare to encounter a fourth-year medical student who is competent to take a history in any type of allergic case, recommend appropriate laboratory studies, and arrive—following correlation of the laboratory tests and the history—at a likely diagnosis. The student's ability to handle the important matter of differential diagnosis in allergic disease is greatly enhanced if he has been well grounded in all the manifestations of the disease, rather than in only those which he has chanced to encounter. Such grounding can be obtained only by studying the management of patients in an allergy clinic, under the supervision of a full-time allergist. Since, at best, the time available for either basic or clinical instruction is limited, the conclusion is inescapable that the instruction of medical students in allergy can be handled most efficiently by a single service. It is for these reasons that we feel it absolutely necessary to have a single instructional coordinating unit which presents the subject of allergy to students as an entity. Wherever this system has been put into practice, it has been received enthusiastically by students and medical staff alike.

The actual curriculum of instruction for the undergraduate medical student should contain provision for a reasonable understanding of the immunological aspects of allergy plus concentration on the clinical entities which allergic disease may produce. The procedures used in diagnosis and treatment should be familiar to the student, but details of these features may well be postponed

until the postgraduate years, during the intern and residency training period. A certain number of formal lectures must be presented in order to cover the subject completely. These should be given in addition to the important bedside teaching with patients. The allergist in charge must know the subject matter covered in these lectures so that he can supplement them with clinical material later.

The University of Michigan Medical School, utilizing two full-time allergy clinics and having access to teaching material from a third clinic, all under the supervision of a full-time allergist on the medical school faculty, is placing considerable emphasis on the teaching of allergic disease. Such instruction has arisen from and been modified by the practical consideration that graduates going out into medical fields have been confronted by cases of allergy and have had no reluctance in reporting back to the medical school any features of their training which were inadequate. It has been necessary to provide practical training in addition to the fundamental theoretical instruction, since many medical graduates are not able to avail themselves of postgraduate training in allergy. There are now approximately twenty accredited hospitals with facilities for postgraduate training in allergy in the United States and, with only about 1,500 full-time physicians practicing clinical allergy, there is great need for additional training sites to assure the future care of the increasing number of allergic patients.

At the University of Michigan the allergy training program is an integral part of the Department of Internal Medicine. While this particular program is not presented as a perfect example, it does provide a basis for comparison. The allergy clinic, with its supervising allergist and its planned program of instruction, produces at the end of four years young physicians who, with some additional experience, can handle patients with competence. And it does provide facilities for residents to obtain additional training in our field. Instruction is spread over a four-year period, but is concentrated in the fourth year. During the freshman year, a single one-hour clinic presentation is devoted to allergy. This hour is scheduled to coincide with the work in the bacteriology department during the period when the freshman student is receiving instruction in basic immunology and immunity. The presentation of this one-hour clinic is supervised by the physician in the allergy section, and he is aware of the fact that, by demonstrating an allergic response in the skin test of a human patient, he is contributing to the interest which the student may have in seeing an anaphylactic reaction produced in a guinea pig in the bacteriology laboratory. As basic science instruction progresses to the second year, the student acquires a familiarity with the pathologic aspects of allergic disease through microscopic study of tissue sections. In the physiology course, during which at least two hours are devoted to lectures on respiratory physiology, he is familiarized with basic concepts of pulmonary function. Patients with disturbance of respiratory function, including asthma, are demonstrated. Again members of the allergy staff are aware of the points covered and are taking care to see that this preclinical material can be applied to patient care as the student reaches the final year of his undergraduate study.

The third-year medical student sees allergic patients in the hospital and in the outpatient departments. It is at this stage that other members of the teaching staff are able to point out the clinical features of allergy. The clinical presentations are supplemented by eight one-hour lectures given by members of the allergy section staff. These lectures cover the theory, etiology, diagnosis and treatment, and other features of the various allergic diseases. In addition, each junior student attends two morning clinics where a patient is presented and discussed, with special emphasis being placed on the allergy history and treatment measures.

In the fourth year, the senior students are all rotated through the allergy clinic, four men at a time. They are assigned to the clinic from 8 o'clock to 12 o'clock for one week, during which time they make daily ward rounds with the allergy staff consultants, observe history-taking, and participate in skin-testing and treatment. Here they have the opportunity to observe the various research projects in allergy which are under way and also to study the photograph files and to discuss the cases of the patients they have seen. While the senior student is on the general medical service, he participates in the care and treatment of allergy patients admitted to the hospital. Again, the staff member from the allergy section advises the student concerning the handling of these patients. Senior students also have at least one clinic period of one hour devoted to discussion of the treatment of asthma. In addition to the formal instruction just mentioned, three hours of lecture demonstration in diet manipulation by a member of the allergy section and a member of the dietetics department are provided for the senior students.

In order to carry out the program that we have instituted at Ann Arbor, it is thoroughly appreciated that there must be a full-time staff assigned to the allergy section and that there must be a clinic which is in operation every day of the week, with staff members constantly available for consultation with students as well as to provide patient care. I fully realize that our program could be improved upon, but at least it is a start toward more adequate training in allergy for undergraduates and a basic background for the education of interns and residents in this very important field of medicine.

A DILEMMA IN ALLERGY

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SINCE the turn of the century, the practice of allergy has undergone phenomenal growth into a distinct medical specialty. The basis of this progress in allergy was the recognition of the clinical significance of the antigen-antibody reaction. A better understanding of immunological mechanisms has helped to clarify many previously obscure diseases, and newer therapeutic measures are now increasingly effective. These facts are well known to you. As a specialty, allergy has kept pace with the other specialties in terms of actual accomplishments and service to the patient. I am sure that many of us feel that allergy has developed more rapidly than some of the other specialties.

It is generally accepted that proper handling of allergic disease is based on careful specific evaluation and maintenance of the same preciseness in therapy. In spite of the promise of so-called "miracle" drugs and hormones to radically modify the allergic constitution, the failure of histaminase, the antihistaminic drugs, and the ketosteroids to substitute for orthodox allergy management is well known. The diagnostic and experimental techniques, the test allergens, the seasonal and geographic pollen and mold calendars, the principles underlying hyposensitization, and, often, adjunctive agents for symptomatic relief from allergic symptoms constitute only a part of the contribution that allergists have made to progress in medicine.

The phenomena of hypersensitiveness involve all tissues of the body and consequently pervade all specialties, be they medicine, pediatrics, otolaryngology, dermatology, gastroenterology, neurology, or hematology. Frequently, allergic emergencies arise and should be recognized in surgical practices, particularly when due to drug or foreign protein reactions. But, regardless of the talent of a physician, the specialty of allergy has grown so complex that it is difficult for me to visualize it being practiced as a side line to another specialty of medicine. Often a physician who is combining another major specialty with allergy will be capable in the former, but will practice only the most superficial allergy, or he may devote his energies to allergy and neglect or give up altogether his other specialty.

The spontaneous emergence of the various medical specialty groups during the last four decades must be considered both cause and effect of revolutionary advances in American medicine. It is of interest to note that the practice of allergy experienced its most rapid growth and matured as a clearly defined specialty during the same period that most of the other specialty groups at-

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tained recognition and established their certifying boards. At present there are nineteen accepted specialty boards in this country, each dedicated to the advancement of its particular field of practice. Yet it is paradoxical and not understandable that separate certification for allergists has not materialized.

Allergy has proved its place among the specialties, and an independent board for certification of allergists is mandatory, in my opinion, for recognition of this fact. Such a board would be in a logical position to advise additional minimal training in related basic sciences, particularly immunology, and in selected special fields of clinical practice, stressing differential diagnosis and techniques of examination so essential to the successful practice of allergy. The board would conduct examinations designed for the complete allergist and thereby assure the maintenance of the highest standards of proficiency. Above all, such a board would be completely unfettered and free to act in the best interest of allergy as an indispensable component of the recognized medical specialties.

It does not matter whether we are entirely in accord with the trend toward specialization, because we cannot deny its benefits in view of the extensive broadening general structure of medical knowledge and practice. At the same time, modern accelerated specialization and creation of the specialty boards have caused a growing fear that individual training programs in many instances are entirely too limited to afford a sufficiently broad collateral background of graduate training and experience. Unquestionably, in our present system of specialty training an alarming tendency is toward an ever narrowing of the experience the average candidate can secure after completing his rotating internship. Fortunate indeed is the physician who elects to go into a specialty program after a few years of general practice in a highly competitive area where his financial success has depended on hard work and serious study of his patients! Obviously, before certification in any specialty, a physician should have an acceptable, broad, well-balanced professional training. I would agree that, insofar as certification in allergy is concerned, prior proficiency in either internal medicine or pediatrics, or both, would afford at least a part of such a background.

Frequently the desirability of formal certification in allergy depends on a point of view. There are now a great many allergists—in fact, a distinct majority—who have traveled the rugged road of experience, many of them even "grown up" with the specialty of allergy, who by every practical criterion are competent specialists. Formal certification would not make them any better allergists. On the other hand, the recent graduate wishing to limit his work to allergy probably will see no other alternative than conforming to the presently prescribed requirements of subcertification. The greater facility for securing adequate fundamental training and clinical experience in the shortest possible time will doubtless compel such decision.

There are about 1,500 allergists in the United States, of whom approximately 200 are subcertified under either internal medicine or pediatrics. A conservative estimate, furthermore, would indicate 15 million allergic persons in the United States, of whom at least one-half suffer from major, often dis-

abling allergic disease. This means that there is only one allergist for each 10,000, or one certified allergist for each 75,000 allergic persons in this country. Assuming that available allergists were evenly distributed according to population density, it is obvious that by far the great majority of allergic patients are treated for their allergic complaints by physicians other than allergists. As a consequence, most allergic diseases are being treated symptomatically, or at best not adequately from a specific standpoint, by general practitioners, or others not well versed in the fundamentals of allergy, often with uncertain or disappointing results. No wonder a good many people despair of satisfactory control for their allergic sufferings and view with skepticism any new approach, even the right one! The implication of these appalling facts is obvious and serves only to remind us that not without cause have confidence and respect from both our colleagues and the lay public as well often been on a precarious footing. Herein may lie at least some explanation for the observation expressed with some concern in various quarters that the specialty of allergy in recent times is not attracting an adequate number of young physicians.

I am glad that public education has been stated as one of the primary aims of the American Foundation for Allergic Diseases, now in its second year of operation. The Foundation will undoubtedly prove to be a powerful enlightening force, not only for the victims of allergic disease, but especially for allergists who must depend on coordinated research to learn more of this baffling malady. But, regardless of the scientific help or professional prestige we can derive from this or any other foundation, there still remains one problem which only we as allergists can and must solve ourselves. I refer to certification in allergy.

In discussing some of the problems arising from the rapid growth of the specialty boards in recent years, Dr. B. R. Kirklin, secretary of the Advisory Board for Medical Specialties, had this to say in 1948[1]: "Some of these maladjustments arise from the difficulty of drawing boundaries between the specialties. Most of these jurisdictional disagreements spring from reasonable differences of opinion, and, though sometimes irritating, are of no great significance." With these statements I can, as an allergist, only partially agree. I am most emphatically opposed to regarding "as of no great significance" the relegation of the specialty of allergy to its present role of a subspecialty of other fields of practice. Yet, at the present time, certification in allergy within the framework of the Advisory Board for Medical Specialties can be accomplished only on a subcertification basis after primary certification in internal medicine or pediatrics. According to this arrangement, an allergist must obtain his certification of proficiency through either parent board, whereupon he would be declared competent to attend any allergic patients who might consult him, regardless of whether he is otherwise fundamentally trained in internal medicine or pediatrics. I do not wish to imply that either "adult" or "general" allergists, as contrasted with those certified as pediatric allergists would use their certification in allergy to invade one another's province of practice. The fact remains that we are trying to subordinate a major medical specialty on an

arbitrary and artificial basis. To my way of thinking, we have to this extent placed the cart before the horse in our present system of certification for allergists.

If I interpret certain trends correctly, we may, in the not-too-distant future, anticipate that the currently increased interest in allergy by many otolaryngologists will result in a clamor by those practitioners for further recognition of their accomplishments in managing allergic problems of the upper respiratory tract. Might this ultimately lead to another effort at subcertification in allergy? How would an "allergist" so classified by such a plan manage bronchial asthma, allergic eczema, allergic gastrointestinal disease, or other manifestations of allergy? Or will we still identify such a specialist as an otolaryngologist capable of handling allergic disease of the upper respiratory tract but insist that he call in one or more other specialists when the allergic process involves additional organs or tissues? It is not too farfetched to extend the same line of thought to include those dermatologists, gastroenterologists, or others who might eventually insist that their interests in the allergic aspects of conditions they ordinarily see be rewarded by special diplomas or other marks of recognition. We undoubtedly would invite an even more ridiculous situation by even thinking of further subcertification in allergy by any presently functioning specialty board.

The allergists of America have declared themselves in favor of a separate board in allergy. I am happy to say that both the American Academy of Allergy and the American College of Allergists are united in a common plea that the practice of allergy be granted this recognition which it so rightfully deserves. I can tell you that in this mutual effort we have made considerable progress and have gained encouraging support in certain quarters. However, I regret to tell you that because such support has not been unanimous we may not expect success in the immediate future. Nevertheless, I pledge to you the continued cooperation of the College in our efforts to establish an autonomous board in allergy, regardless of whatever additional steps we may be forced to take.

REFERENCE

1. Kirklin, B. R.: Virginia M. Monthly **75**: 597, 1948.

HISTORIAN'S REPORT

The eleventh annual meeting of the American Academy of Allergy was held at the Hotel Statler, New York, New York, Feb. 7, 8, and 9, 1955. The total registration was 685, with 213 fellows, 233 members, and 239 guests attending. In addition, there were 193 exhibit personnel staffing forty-two commercial exhibits.

On February 4, 5, and 6, a postgraduate teaching program was conducted at the Roosevelt, New York, and Mt. Sinai Hospitals concerning contact dermatitis and asthma, with a final panel discussion. This excellent program was attended by capacity numbers.

The scientific program given on February 7, 8, and 9, consisted of twenty-six papers representing original investigative work in the various fields of clinical allergy. The Ciba Seminar on Recent Advances in Hypersensitivity, with Dr. S. M. Feinberg as chairman, was held on Monday, February 7. This seminar will be published in the JOURNAL OF ALLERGY.

At the morning session of February 8, Dr. Homer E. Prince, as the president of the American College of Allergists, presented an address entitled "Common Sense Allergy."

The Presidential Luncheon which followed at noon was of great historical interest. On this occasion Dr. John M. Sheldon, the president, presented an engraved gavel to each of the past presidents of the Academy and the parent organizations, The Association for the Study of Asthma and Allied Conditions and The American Association for the Study of Allergy. Those able to attend to receive this honor were Drs. Horace S. Baldwin, Harry S. Bernton, J. Harvey Black, Stearns S. Bullen, Walter S. Burrage, Robert A. Chobot, Milton B. Cole, Robert A. Cooke, Leo H. Criep, Charles H. Eyermann, Samuel M. Feinberg, Leslie N. Gay, Sanford B. Hooker, Harry L. Huber, Richard A. Kern, M. Murray Peshkin, George Piness, Francis M. Rackemann, Ben Z. Rappaport, Will C. Spain, Theodore L. Squier, Oscar Swineford, Jr., Louis Tuft, Matthew Walzer, and Walter L. Winkenwerder.

At the International Luncheon, eight countries in addition to the United States were represented.

At the Business Meeting, held in the afternoon of February 8, Dr. Francis M. Rackemann was elected an honorary fellow of the Academy; thirty members were advanced to fellowship; two affiliate fellows were elected; and forty-six new members were elected. Dr. Carl E. Arbesman was designated president-elect. In addition, the following officers were elected: vice-president, Dr. Abraham Colmes; secretary, Dr. Francis C. Lowell; treasurer, Dr. Max Samter; historian, Dr. Homer A. Howes; member of the Executive Committee, Dr. Bram Rose. Dr. E. Brum Negreiros, chairman of the Organizing Committee of

Brazil of the Second International Congress of Allergology, invited all members of the Academy to attend the Second International Congress of Allergology to be held in Rio de Janeiro next November. Dr. Stanley F. Hampton, St. Louis, Missouri, assumed the Presidency of the Academy.

Throughout the meeting the Hostess Committee, under the co-chairmanship of Mrs. Horace S. Baldwin and Mrs. Selian Hebald, provided extremely valuable services for which the Academy is most grateful.

The New York Allergy Society gave a reception for the members, their guests, and wives in the Starlight Room of the Waldorf-Astoria on the evening of February 6, and the Schering Corporation was host at the annual cocktail party at the Hotel Statler on the evening of February 7.

The commercial and scientific exhibits were of exceptional interest. The American Foundation for Allergic Diseases was represented here.

The twelfth annual meeting will be held in St. Louis, Feb. 6, 7, and 8, 1956.

The following members were advanced to fellowship at the annual meeting:

Ralph Bookman
Maclyn Cagan
Philip A. Cavelti
Cecil Collins-Williams
Bernard T. Fein
Claude A. Frazier
John L. Guerrant
Helen C. Hayden
DeWitt H. Hotchkiss
Thomas G. Johnston
Walter R. Kessler
Leopold C. Lazarowitz
Jacques Leger
Walter R. MacLaren
Saul Malkiel
C. James Malloy
Meyer B. Marks
Lloyd Dewald Mayer
Joseph W. Noah
Ely Perlman
Jack Rom
Nathan Schaffer
Paul M. Seebohm
Edward E. P. Seidman
Joseph H. Shaffer
Maurice H. Shulman
Gadiel M. Smith
Milton J. Steinhardt
George W. Warrick
Ross R. Weller

The following physicians were elected to affiliate fellowship:

Ludwig A. Sternberger
Frank J. Dixon, Jr.

The following physicians were elected to membership:

Stanley S. Avren
H. S. Barlow
Noah Barysh
John F. Beakey
Harold Bedell
Adolph H. Blier
S. Gerald Booke
John H. Burger
Ben C. Eisenberg
Clifford P. Powell
Howard G. Rapaport
Joseph Rebhun
Norman B. Richard
George Rosenfeld
Jean Roy
T. Harry Saferstein
Arthur S. Sandler
Ines Maria H. Santos

Cutting B. Favour
Max W. Flothow
John K. Fulton
Robert J. Gaspich
George Gittelson
Mathilde May Gould
William B. Greenberg
Joseph D. Howell
Wilfred Leith
Mary F. Lerner
Edward D. Loweecey
William M. Mount
Robert D. O'Connor
Isadore Pitesky
Kenneth L. Sears
A. Harvey Simmons
David A. Stadtner
John R. Talbot
Grace Talbott
Hugh Tatlock
Donald L. Thurston
Henry Turkel
John Vaughan
Sherman P. Vinograd
Leonard Wolin
Reynaldo Araujo Cintro
Carlos Benaim-Pinto
Eduardo R. Pons, Jr.

Following is a list of the officers for 1955:

Stanley F. Hampton, president
Carl E. Arbesman, president-elect
Abraham Colmes, vice-president
Francis C. Lowell, secretary
Max Samter, treasurer
Homer A. Howes, historian
Bram Rose, member of the Executive Committee.

HOMER A. HOWES, Historian.

Announcement

Jewish National Home for Asthmatic Children

The Jewish National Home for Asthmatic Children announces a one-year fellowship in pediatric allergy, approved by the subspecialty Board of Pediatric Allergy. The stipend is $3,000.00 to $3,600.00 per annum, which is deemed to be free of income tax.

Address applications to Harold S. Tuft, M.D., Medical Director, Jewish National Home for Asthmatic Children, 3447 West 19th Ave., Denver 4, Colorado.